1939

Third Annual Report

U. S. Regional Pasture Research Laboratory

State College, Pennsylvania.

Division of Forage Crops and Diseases

U.S.

Bureau of Plant Industry

and

The Agricultural Experiment Stations

of the

Northeastern States

Cooperating.

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Thirty-five copies of this report were made and distributed as follows:

Three copies to the Division of Forage Crops and Diseases; one copy to each of the twelve Directors of the cooperating State Agricultural Experiment Stations in the Northeastern United States; one copy to the President of the Pennsylvania State College; one copy to the Director of each of the following State Agricultural Experiment Stations: Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Tennessee, Virginia, and Wisconsin; one copy to the Dominion Agrostologist, Ottawa, Canada; and the remaining four copies to the U. S. Regional Pasture Research Laboratory.

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This annual report of activity at the Pasture Laboratory, as well as of that at the State stations with which the Laboratory cooperates, is a progress report and as such may contain statements which may or may not be verified by subsequent experiments. The fact that any statement has been made herein does not necessarily constitute publication. For this reason citation to particular statements in the report should not be published, unless permission has been previously granted by the Laboratory or the State station concerned.

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REPORT OF

THE UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY

For the Calendar Year,

January 1, 1939 to January 1, 1940.

INTRODUCTION

During 1939 the research program of the U. S. Regional Pasture Research Laboratory has been carried forward essentially as agreed upon by representatives of the twelve northeastern Agricultural Experiment Stations and the United States Department of Agriculture. Some results have been obtained of sufficient interest to warrant publication. Significant facts have been discovered relative to the inheritance of sterility in white clover, Trifolium repens; to the effect of chromosome doubling on chemical composition of ryegrass, Lolium perenne; to the effect of supplementary light and low temperatures (centrigrade scale) in inducing flowering of pasture grasses and legumes in the greenhouse; to chromosome behavior in certain species of grasses; to the occurrence of Helminthosporium turcicum in seed of Sudan grass, Sorghum vulgare var. sudanense; to the variation of calcium, phosphorus, and potassium among individual plants of white clover; to the comparative ability of individual plants of white clover, Trifolium repens, of orchard grass, Dactylis glomerata, and of Kentucky bluegrass, Poa pratensis, to form sods when clonally increased; to the comparative response of four clones of Kentucky bluegrass when grown in nutrient gravel cultures at two levels of nitrogen and under various clipping treatments; and to developing suitable chemical methods for determining the hydrocyanic acid contents of white clover and the carbohydrate fractions of certain grasses.

In reporting progress of research activity at the Laboratory, it was thought advisable only to summarize the results obtained during the year and in some cases to give conclusions reached rather than to include the bulk of the substantiating data. It is hoped that this plan did not seriously impair the usefulness of the Report as it certainly made it less bulky and perhaps more readable. Suggestions are always gratefully received.

Definite progress was made during the year in developing cooperative research. Two important conferences were held at State College, namely, the plant breeding conference in June and the collaborators' meeting in October. As a more or less direct outgrowth of the plant breeding conference three specific cooperative projects were developed between certain State stations and the Laboratory. It is expected that additional similar projects will be developed in the near future.

The appendix again, as last year, contains a progress report of pasture research at the twelve State agricultural experiment stations with which the Laboratory is cooperating. There are also appended somewhat detailed reports of the plant breeding conference and the annual collaborators' meeting.

An attempt has been made to index the State reports. This, together with the somewhat extended "Table of Contents", should facilitate locating specific subjects discussed.

CHANGES IN PERSONNEL

During the year three members of the Laboratory staff resigned. W. H. Brittingham, assistant cytologist, and J. G. Conti, technical assistant in biochemistry, enrolled last September at the University of Maryland to resume graduate studies. G. H. Ahlgren, technical assistant, resigned December 1 to accept an appointment as graduate assistant in the Agronomy Department of the New Jersey Agricultural Experiment Station. Armand G. Morin, who received his training in biochemistry at the University of New Hampshire, was appointed as technical assistant on September 1.

One change has been made in the personnel of the collaborators. Pursuant to a recommendation of the Maine Agricultural Experiment Station, D. S. Fink, who has charge of the pasture investigations at that station, was appointed collaborator in the place of J. A. Chucka.

As this is being written, word had just reached us of the untimely death of Director J. E. Metzger. He has been one of the most helpful and staunchest supporters of regional pasture research. We sincerely regret his passing.

ADDITIONS TO BUILDINGS AND EQUIPMENT

During the year miscellaneous small tables and shelves, such as are usually needed in greenhouses and laboratories, were built. Twelve suitable bee cages were constructed for use in connection with the clover pollination studies. A mouse proof room for storing plant material was completed in the attic of the headhouse.

The temperature control equipment was augmented by converting a meat show case into a refrigerator with three compartments. The three compartments, which admit ordinary daylight in the greenhouse, have a temperature range of -5° to 40°C. and are controlled independently. It is planned to use this equipment in connection with life history studies of plant pathogens.

An apparatus for integrating daylight was constructed by a member of the Laboratory staff in collaboration with a member of the Electrical Engineering Department of the Pennsylvania State College. This apparatus, together with the light intensity recorder transferred to the Laboratory by the Division of Forage Crops and Diseases, constitute valuable additional equipment for studying plant-light relations.

ACTIVITY RELATING TO COORDINATION OF RESEARCH

As in previous years, considerable thought was given to ways and means of bringing about closer working relations among all persons

actively interested in pasture research in the Northeastern United States. Most of the activities directed toward the accomplishment of this purpose cenetered around or developed as a result of the collaborators' annual meeting and the meeting of the plant breeders.

Meeting of Collaborators

The collaborators' annual meeting was held October 3 and 4, 1939, at State College, Pennsylvania. Inasmuch as a detailed report is given in Appendix B, only brief mention of some of the more important features and consequences of the meeting will be made here. The meeting differed from the previous ones in that most of the discussions centered around pasture research programs of the State stations rather than that of the Laboratory. A uniform plan was adopted for making State progress reports to be included in the annual report of the Laboratory. The research conducted at the Laboratory since the previous meeting of the collaborators was briefly presented by the project leaders.

A feature of this third annual meeting was the consideration given by the group to new pasture research projects being developed or contemplated in the immediate future. Seven such projects were presented and discussed. As a more or less direct outgrowth of these discussions, a conference of representatives of the Pennsylvania and Cornell Agricultural Experiment Stations and of the Division of Forage Crops and Diseases was held on October 25 at Montrose, Pennsylvania, to give further consideration to a rather extensive pasture management study to be developed there by the Pennsylvania station and the Division.

Another concrete result from the discussion of contemplated pasture projects was an agreement to circulate the outlines of such projects while they were still in a formative stage among the collaborators for suggestions and criticisms. Since the collaborators' meeting was held two project outlines have been circulated, one originating at the Delaware station and one at the Rhode Island station.

Just before adjournment the collaborators gave some consideration to a suitable program for the 1940 annual meeting. It was suggested that a program be developed around one or more topics and that each collaborator, insofar as practicable, bring with him to next year's meeting his experiment station associates who may be particularly interested in the topics to be discussed.

Meeting of Plant Breeders

As a sequel to the meeting of plant breeders held last year in New York City, a second one was held June 27 at the Pasture Laboratory, State College, Pennsylvania. Representatives were present from seven States. A more or less detailed report of the meeting may be found in Appendix C.

The group expressed themselves as favoring the idea of each station limiting its major breeding activity, insofar as pasture plants are concerned, to one or two species. It was agreed that the primary responsibility for the actual breeding of improved strains should be assumed by the State stations. However, this work should be coordinated and in some cases it may be desirable to set up cooperative projects either among State stations or between State stations and the Laboratory. A number of delegates indicated that additional technical assistance, such as provided by graduate students or technicians, would be particularly helpful in carrying on an aggressive breeding program. Proposed cooperative breeding projects between certain State stations and the Laboratory were discussed.

Development of Cooperative Projects

Since the plant breeding conference was held, three projects, outlining a cooperative attack on the improvement of pasture plants by breeding, have been prepared and others are in prospect. The titles of the project outlines already developed and the cooperating agencies are as follows:

Title: Breeding Kentucky Bluegrass (Poa pratensis) for Improved Pasture Types.
By: Pennsylvania Agricultural Experiment Station and the Laboratory.

Title: Selection, Inbreeding, and Crossing to Obtain Orchard Grass (Dactylis glomerata) Strains Adapted Particularly for Pastures in Maryland.
By: Maryland Agricultural Experiment Station and the Laboratory.

Title: Breeding White Clover for Pastures.
By: New Jersey Agricultural Experiment Station and the Laboratory.

In addition to the breeding projects, one concerned with a plant disease has been outlined as follows:

Title: Investigations of Snowmold on Pasture and Fine Turf Grasses.
By: Pennsylvania Agricultural Experiment Station and the Laboratory.

Miscellany

The informal cooperation of the New Hampshire station and the Laboratory in a study of the chemical composition of certain pasture grasses mentioned in the last annual report has been continued on an informal basis.

During the year there were something over 100 visitors at the Laboratory, most of them agronomists professionally interested

in pasture research. These visitors came from five foreign countries and twelve States in addition to the twelve States with which the Laboratory is cooperating.

RESEARCH AT THE LABORATORY

The research activity at the Laboratory is discussed under cytogenetics and breeding of grasses, cytogenetics and breeding of legumes, physiology and composition of pasture plants, and pathology. This grouping is followed because the main research activities are represented and it is hoped that the arrangement will make readily accessible the contents of the Report. Much of the research described has been carried on cooperatively between two or more members of the Laboratory staff but in only a few cases has the cooperation been pointed out.

Cytogenetics and Breeding of Grasses

Fertility Studies in Dactylis glomerata, Lolium perenne, and Phleum pratense

Emasculation with Hot Water

The studies reported in the 1938 Annual Report, page 15, have been continued, using three plants of timothy and two of orchard grass. The results with timothy agree in general with those reported. Treatments with water at 44°C. do not appear to completely inactivate the pollen. Heads treated at 46° and 48° did not set seed when selfed but set a normal amount of seed when untreated pollen was applied. The results with orchard grass were so variable that conclusions cannot be drawn from them.

Variation and Inheritance of Ability to Set Seed Under Bag

Studies of the variation and inheritance of ability to set seed under bag are being made, using the clonal rows and first inbred generation progenies of the selected plants of orchard grass, timothy, and perennial ryegrass. (See 1938 Annual Report, page 23, for method of selection and planting). A single bag was placed on panicles of one plant in each clonal row in each replication and of each inbred plant that flowered. All inbreds of timothy and perennial ryegrass and about half of the inbreds of orchard grass flowered. In addition to this material, four first generation inbred families of orchard grass in their second year of growth were available and from two to six bags were placed on panicles of each of these plants. Seed set was expressed as number of seeds per panicle and was determined by dividing the number of seeds per bag by the number of panicles enclosed in that particular bag.

Data were obtained for all three replications of 54 of the orchard grass clones and these data were analyzed by the analysis of variance, separating variance into that due to replications, clones, and error. The value of F for comparing mean square for replications with mean square for error was greater than the value of F for P of 0.01. The mean numbers of seeds per panicle for the three replications were 30.98, 75.87, and 69.56.

The 54 clones ranged in mean number of seeds per panicle from 0.3 to 288. Since it is expected that error variance will be higher for those plants with higher seed setting ability, the 54 clones were separated into four classes and an analysis of variance calculated for each class. The data for the classes are presented in Table 1.

Table 1.- Standard error for replicated clones of orchard grass differing in mean number of seeds per panicle.

Range in number of seeds per panicle	Number plants	Average number of seeds per panicle	S. E. of single*
0.3 to 288	54	58.80	57.58
0.3 to 25	20	7.58	9.80
25.3 to 50	11	37.94	33.25
50.3 to 100	10	64.43	54.02
100.3 to 288	13	150.92	87.90

* Calculated from mean square for error.

The variance due to error in this experiment is a composite of variation between and within plants. No estimation of variance within plants is available in this case since only one bag was placed on each plant. Such information can be obtained, however, from the two-year old inbred plants on each of which more than one bag was used. These data are given in Table 2.

Table 2.- Standard error within plants of inbred progenies, differing in mean numbers of seeds per head.

Range in number of seeds per panicle	Number plants	Average number of seeds per panicle	S. E. of single*
0 to 122.5	81	21.27	23.35
0 to 141.25	39	41.09	24.73
0.75 to 188.0	80	50.48	33.42

* Calculated from variance within plants.

Although the standard errors in this experiment are not as large as those for the replicated clones, it is apparent that variation within plants contributes a considerable amount to the variation between plants. This portion of the error variance can be reduced by increasing the number of bags per plant. A manuscript reporting the data on variation in seed setting in orchard grass has been prepared. (See page 57).

The variation between plants was of similar magnitude for plants of timothy and perennial ryegrass with comparable mean numbers of seeds per inflorescence.

The differences between the average seed set of all of the clonal rows in 1939 and the parent plants in 1938 were not statistically significant in orchard grass and timothy. In perennial ryegrass the average was significantly lower in 1939; the mean seed set in 1938 was 12.22 and in 1939 it was 5.25 seeds per inflorescence. In orchard grass, the correlation coefficient for seed set in 1938 and 1939 was 0.55 with 58 degrees of freedom; in perennial ryegrass it was 0.39 with 53 degrees of freedom; and in timothy it was 0.20 with 58 degrees of freedom.

Preliminary information on inheritance of self-fertility in orchard grass was obtained from the data collected from the two-year old inbred progenies. The parent of one of these progenies, 37 OG 11(3), was almost completely self-sterile. No seed was obtained under bag on this plant in the field in 1937, 1938, or 1939, but four seeds were obtained from two panicles of the plant in the greenhouse in the winter of 1937-38. Of two plants obtained from these seeds, one set no seed in 1939 on 26 bagged panicles, while the other averaged four seeds per panicle for three bags.

In the other three inbred families, containing 39, 80, and 81 plants, the plants varied in average number of seeds per panicle from zero or less than one to over 100. Data on seed set of the parents of two of the inbred families were obtained and in each case the average seed set on the inbreds was lower than that of the parent. The continuous variation in each inbred family suggests a somewhat complicated genetical basis for self-fertility in orchard grass.

The inbred plants of orchard grass which were interplanted with the clones and which were in their first year of growth set few seeds under bag. Seed set was also poor in most inbred families of timothy and perennial ryegrass. It is possible that data on seed setting ability which are collected from plants in their first growing season are not reliable as a measure of the self-fertility of those plants.

Inheritance of Male Sterility

In the F_1 of crosses involving one male sterile parent of perennial ryegrass with three male fertile plants, 49 plants were classified for male sterility. On the plants designated as male sterile, the anthers failed to dehisce until several hours or a day after excertion. At that time little or no pollen was shed, the rupturing of the anthers apparently resulting primarily from drying. Whether any viable pollen was produced by these plants has not been determined. Fourteen plants of the 49 were classed as male sterile and 5 as intermediate. Progeny tests, using seed produced under bag on some of the normal plants and from pollination of plants classed as male sterile with pollen from normal

plants, are being made to obtain more complete data on the inheritance of this character.

Forty plants of an F_1 of male sterile x male fertile orchard grass were examined and 8 were classed as male sterile, while 4 were intermediate. The F_2 generations from selfed seed from male fertile plants and from seed produced by pollinating male sterile with pollen from male fertile plants have been established for use in further studies of the inheritance of male sterility. The male sterile parent of this cross and six other male sterile plants have each been crossed with from one to eight male fertile plants and the F_1 progenies have been planted.

One male sterile timothy plant has been crossed with two male fertile ones and another male sterile with six male fertile ones and the F_1's have been planted in the field.

Varietal Improvement of Dactylis glomerata, Lolium perenne, Poa pratensis, Phleum pratense, and Sorghum vulgare var. sudanense

Inbreeding

Observations on the first inbred (I_1) generation families of orchard grass, perennial ryegrass, and timothy (see 1938 Annual Report, page 23), indicate that reduction in vigor has resulted, the degree of reduction differing for the different families. On the average, perennial ryegrass and timothy showed more reduction than orchard grass. All surviving plants in the three species were sufficiently vigorous to permit further inbreeding. Although segregation occurred in all I_1 families, the similarity of plant type of the inbreds and their parents was striking.

Spaced plant nurseries have been established from several seed collections and introductions of timothy and perennial ryegrass; these plantings to be used as additional source material for breeding and cytogenetic investigations. First generation inbred families have been planted from 12 selected plants of orchard grass and 25 selected plants of perennial ryegrass. From selected I_1 plants, 52 I_2 families of orchard grass and 19 I_2 families of perennial ryegrass were planted.

The progenies of Kentucky bluegrass plants varied from those in which all plants appeared identical with the parent to those in which a majority of the plants differed from the parental type. Most of the progenies tended to approach the former type of breeding behavior. From the isolations from old pastures (see 1938 Annual Report, page 43) 12 plants were selected and progenies of these have been planted to test their breeding behavior.

Inbreeding has been continued in Sudan grass. Over 250 lines have been inbred three or more years and about 150 lines have been inbred two years.

Effects of Crossing Heterozygous Plants From Open Pollinated Seed

Preliminary to the combination of plants into strains, investigations are being initiated on the questions of (1) degree of heterosis to be expected from crossing heterozygous plants, (2) differential combining ability if heterosis does occur, and (3) methods of estimating combining ability without resorting to diallel crosses. Preliminary data from F_1's of perennial ryegrass plants from open pollinated seed indicate that heterosis results from certain combinations. Diallel crosses of 10 heterozygous orchard grass plants and 10 heterozygous timothy plants have been planted, the material to be used for measuring the degree of heterosis and differential combining ability.

Evaluation of Plant Types

The plots from clonal increases of single plants were clipped periodically throughout the growing season. Yield samples were taken from the orchard grass plots at each clipping and from the Kentucky bluegrass plots at only one clipping, on June 6. The yields of some of the plots of orchard grass were more than double the yields of other plots throughout the season. Wide differences in yield were obtained also from the Kentucky bluegrass plots.

Observations were made of the plots at intervals during the growing season, recording percentage of clover, percentage of ground covered by the grass, and any other notes of interest. Differences in percentage of clover in different plots was apparent but whether this was an effect of the grass strain could not be established because of wide variation in different replications. Records on spreading ability, type of sod and other characters checked well with the data taken from observations of the individual spaced plants in the original nursery.

Because of the labor involved in handling so many plots, it was decided to concentrate efforts on fewer species. Since the Canada bluegrass and red top plots had made such poor showing, the plots of these species have been discarded.

Genetical Investigations

Inheritance of Rust Reaction in Dactylis glomerata Lolium perenne, and Phleum pratense

The rust reaction of plants of 76 I_1 families of orchard grass and of the parental clones of 60 of these families was classified. Three I_1 families had only resistant plants, seven only susceptible, and 66 were segregating. Of these, 29 had a

preponderance of resistant plants and 37 had a preponderance of susceptible plants. No measures had been taken to increase the natural epiphytotic and the uniformity of distribution of the inoculum was subject to question. Conclusions regarding the cytogenetic basis of rust reaction must await data from further experiments.

The parental clones and I_1 progenies of 58 families of perennial ryegrass were classified for resistance to a natural epiphytotic of crown rust. Of the parental clones, 23 were classed as resistant and 35 as susceptible. All progenies of susceptible parents were susceptible. Five of the resistant parents had only resistant progenies, while the progenies of the other 18 resistant parents were segregating for rust reaction.

In timothy, only two of the 60 parental clones showed any resistance in the natural epiphytotic of stem rust. These two plants had numerous resistant type pustules, while all other parents had large susceptible pustules that killed the leaves in most cases. Susceptible plants had generally susceptible progenies, while the progenies of the two resistant plants were segregating for the parental type of resistance and susceptibility.

Tetrasomic Inheritance in Dactylis glomerata

The occurrence of quadrivalents at meiosis in orchard grass indicates that tetrasomic inheritance should obtain. When self-pollinated, one plant produced normal green and chlorophyll deficient (lethal) seedlings in a ratio approximating 35:1. In progeny tests of 63 I_1 plants, 16 produced only normal seedlings and 47 produced both normal and chlorophyll deficient seedlings. By means of the X^2 test the segregating families were separated into 32 approximating 35:1 and 15 approximating 3:1 ratios. Assuming that the parent plant was duplex (AAaa) with the gene located near the spindle fiber attachment, the expected ratio is 16.2 not segregating, 32.4 segregating 35:1, and 14.4 segregating 3:1. Chi-square for fit of observed to calculated gave a value of P above 0.99. These data have been published (see page 57).

The inheritance of a number of other characters is being investigated (see 1938 Annual Report, page 31), but final data have not been obtained. However, the evidence to date substantiates the above conclusion, i.e., that tetrasomic inheritance obtains in orchard grass.

Heritable Characters in Lolium perenne

Insufficient seed has been obtained from the I_1 plants to permit use of the progeny test in determining more accurately the mode of inheritance of the characters reported in the 1938 Annual Report, pages 30-31. Second generations of intercrosses

of plants obtained from seed produced on one plant are segregating for red vs. green coleoptile, green vs. light green plant color, decumbent vs. erect growth habit, normal vs. dwarf plant type, normal vs. abnormal leaf emergence, perennial vs. annual habit, awned vs. awnless florets, and red vs. green adult plant color. The character pairs are listed in order of dominance, insofar as that has been determined. Some of these characters appear clearly monofactorial in inheritance; for the others the results are still uncertain.

Two new characters, both apparently conditioned by single recessive factors, were found in I_1 families. One was manifest by a poorly developed, panicle-like inflorescence with aborted florets; the other by a considerable proliferation of florets giving the spikelets an overgrown appearance, particularly on the spikes which emerged early.

Comparison of Diploid and Induced Autotetraploid Lolium perenne

Comparative studies of chemical composition of diploid and tetraploid perennial ryegrass are summarized elsewhere in this report. Morphological and fertility investigations have been conducted using material grown in the greenhouse. The tetraploids have broader, thicker, and longer leaves, thicker tillers and culms, more robust-appearing inflorescences, larger anthers and florets, and larger seeds. (See Figure 1, A). The diploids tiller more profusely and consequently have a larger number of leaves. Seed set was poor in the greenhouse but the limited data indicate that the tetraploids were somewhat reduced in fertility as compared with the diploids. No significant differences were found in date of initial flowering.

In root tips the cells were larger in the tetraploids. In cross sections of the leaves, no striking differences in cell size were apparent although variation in size of cells in the same leaf made comparisons difficult. The tetraploids had, on the average, one more vascular bundle per leaf and the diameter of the xylem was slightly greater in the tetraploids. Such great differences in stomata size as has been consistently reported for polyploids as compared with diploids have not been found in this material. In samples taken near the base of the leaf, the stomata of the tetraploid were found to be slightly, but perhaps not significantly, smaller. In samples from near the tips of the leaves the stomata of the tetraploids were slightly larger. No differences were found in size of chloroplasts in the leaves.

Diploid and tetraploid isolations from the same plants are now being grown in the field where more comparative data can be collected.

A paper reporting the methods used in producing tetraploids in Lolium perenne has been published in the Journal of Heredity. (See page 57).

Cytological Investigations

Chromosome Behavior and Aneuploidy in Naturally Occuring Autotetraploids, Agropyron cristatum, Arrhenatherum elatius, and Dactylis glomerata

The chromosome numbers of 116 plants of orchard grass have been determined from root tips. Twenty-two percent had 27 chromosomes, 59 percent 28, 12 percent 29, and 8 percent 30. One plant out of ten in tall oat grass had 27 chromosomes, and one out of six in crested wheatgrass had approximately 31 chromosomes, while the rest were normal (2n = 28).

The number of quadrivalents per microsporocyte varied from one to seven in each species at diakinesis and metaphase I. For three plants of orchard grass the range in average quadrivalent frequency was 3.3 to 4.2 with an average of 3.7; for five plants of crested wheatgrass it was 3.4 to 4.2 with an average of 3.7; and for nine plants of tall oat grass it was 3.4 to 4.6 with an average of 3.8. The percentage of ring and chain quadrivalents showing an alternate type of orientation at metaphase I varied from 61.8 to 90.7 among four plants of tall oat grass and was 79.1 and 90.6, respectively, for two plants of crested wheatgrass. The plants of each species varied in percentage of sporocytes with one or more univalents at metaphase I. The lowest frequency was none in 184 sporocytes examined in one plant of tall oat grass, while the highest frequency was 32.3 percent in one plant of crested wheatgrass.

Lagging univalents at anaphase I divided equationally and the daughter half-chromosomes moved towards the two poles. The frequency of lagging univalents in different plants was correlated with the frequency of their metaphase I nuclei showing unpaired chromosomes, but in most plants there was an excess of laggards, indicating that some of them originated from other sources than metaphase I univalents. The number of micronuclei in the sporocytes at interphase I was lower than expected from the number of lagging univalents at anaphase I, suggesting that a majority of the half chromosomes arrived at the poles in time to be included in the daughter nuclei. Dicentric chromatic bridges and acentric fragments were found in some sporocytes in anaphase I, telophase I, or both stages, in all plants studied. It is assumed that these bridges and fragments originated from crossing-over in areas heterozygous for an inversion. The presence of bridges and fragments at anaphase II in certain sporocytes supports this assumption. Three plants showed one or more sporocytes with two bridges plus two fragments, indicating that they may have been heterozygous for two inversions.

The percentage of quartets showing micronuclei was correlated in the different plants with the frequency of sporocytes showing lagging univalents at anaphase I. Observed numbers of micronuclei in the quartets from eight plants varied from 13 to 52 percent of the expected numbers which were calculated on the assumption that all laggards formed micronuclei and none were included in the quartet nuclei. One plant had more than was expected and may have been a valid exception.

The types of quartets, based on number and position of micronuclei, were predicted for sporocytes having 1, 2, and 3 lagging univalents at anaphase I on the assumption that micronuclei originated only from the loss of the daughter half chromosomes of the lagging univalents. Plants having a maximum of 1, 2, and 3 lagging univalents per sporocyte, respectively, showed only the types of quartets expected on the hypothesis. One plant showed more complicated types of quartets and this plant had a maximum of 8 lagging univalents. The results indicate that most, if not all, of the micronuclei in these plants originated from lagging and dividing univalents at anaphase I.

This material was presented before the Genetics Society of America at the Columbus meetings of the American Association for the Advancement of Science and a manuscript summarizing these studies has been prepared (see p.57).

Meiotic Behavior and Chromosome Morphology in Diploid Lolium perenne

Studies of chromosome morphology in prophase of the first post-meiotic mitosis in microspores of perennial ryegrass have been continued. Further confirmation of the results reported in the 1938 Annual Report, pages 40-41, has been obtained. A few measurements have been made in mid-prophase of the first division of meiosis and the interchormosomal ratios and arm length ratios agree well with the ratios obtained in the microspores.

Studies of various stages of meiosis have been made using 19 plants from open pollinated seed of perennial ryegrass. Chiasma frequency and number of open bivalents were determined for 15 to 64 metaphase I sporocytes in these plants. The average number of chiasmata per sporocyte varied from 10.5 to 14.8 for the different plants, while the average number of terminal chiasmata ranged from 8.8 to 12.9 per sporocyte with the terminalization coefficient varying form 0.77 to 0.93. The average number of open bivalents per sporocyte was correlated with the chiasma frequency as expected, but the association was not complete. The average ranged from 0.93 to 4.21.

From 68 to 360 metaphase I sporocytes from each plant were examined for presence of univalents, non-orientated bivalents, and loosely attached bivalents. One plant had no univalents in 212 microsporocytes, two plants had a maximum of 4 univalents and 16 plants had a maximum of 2 univalents. The range in percentage of sporocytes having univalents was 0 to 9.7 percent. All plants showed some sporocytes with one or more non-orientated bivalents and with one or more loosely attached bivalents. The range in percentage of sporocytes showing the two irregularities was 1.3 to 14.8 and 1.0 to 32.0, respectively.

All univalents which were classified as lagging at anaphase I were split longitudinally and in some cases the chromatids had already separated and were moving towards the two poles. From 61 to 228 anaphase I sporocytes were examined in each plant. No lagging univalents were found in sporocytes of four plants, while

the remaining plants varied from 0.5 to 41.6 percent of the sporocytes with one or more lagging univalents. One plant had a maximum of six lagging univalents per sporocyte. The presence of lagging univalents at anaphase I was correlated with the presence of unpaired chromosomes at metaphase I, indicating that the metaphase I univalents tended to lag at anaphase I. Laggards at anaphase I also originated from some other source, at least in some plants. The plant with 41.6 percent of sporocytes with lagging chromosomes at anaphase I had only 2.1 percent of the metaphase I sporocytes with univalents.

In this material, no correlation was found between non-orientated bivalents or loosely attached bivalents at metaphase I and lagging chromosomes at anaphase I. It was expected that non-orientated bivalents might cause irregular distribution at anaphase I. However, the numbers of chromosomes in the two groups at this stage were determined in 633 sporocytes in which laggards did not occur and in all cases the distribution was 7 - 7.

Dicentric chromatin bridges and acentric fragments, interpreted as resulting from crossing-over in inversions, were found in some sporocytes of 12 of the 19 plants. Presence of bridges and fragments in some anaphase II figures in these plants supports this interpretation. In another plant, 30 to 50 percent of the sporocytes showed a chromatin bridge at anaphase I. This bridge separates in most sporocytes so that telophase I figures showing a bridge are rare. No fragment has been found associated with this bridge and bridges have not been found at anaphase II. Therefore, it is doubtful whether this plant is heterozygous for an inversion. Instead, the character of the bridge suggests the delayed terminalization of a chiasma.

The number of micronuclei at telophase I was lower than expected if all chromatids from lagging anaphase I univalents were lost, indicating that a majority arrived at the poles in time to be included in the telophase I nuclei. Comparisons of the percentage of micronuclei at telophase I with the percentage at prophase II and metaphase II, indicated that they did not disintegrate and were not included in the prophase II nuclei.

Comparison of the frequency of micronuclei in the quartets with the percentage of sporocytes showing lagging univalents at anaphase I indicated that the daughter half chromosomes tend to be lost either at telophase I or telophase II and thus form micronuclei. Nevertheless, a considerable number of them are included in the quartet nuclei. The data further indicated that micronuclei were produced also in some plants from other sources than anaphase I laggards, since types of quartets were found frequently which could not be explained on the basis of behavior of these laggards.

Aneuploids are rare in the populations of perennial ryegrass that have been examined, none having been found in more than 100 plants from open pollinated seed. The presence of lagging and dividing univalents at anaphase I should contribute to the formation of aneuploid gametes and, consequently, aneuploid plants. The chromosome numbers have been determined for 52 progenies of the plant having 41.6 percent of anaphase I sporocytes with laggards and four of the plants were aneuploid.

Irregularities in premeiotic mitotic divisions appear to occur in some plants. In plants with a majority of normal (2n) sporocytes, sporocytes have been found which were 2n - 1, 2n + 1, 2n + 2, 2n + fragments, and 4n.

A manuscript summarizing these data has been prepared (see page 57).

Meiotic Behavior in Triploid Lolium perenne

One triploid plant was obtained from pollination of a tetraploid spike with pollen from a diploid plant. Association of chromosomes in prophase is in pairs. Therefore, with only homologous association, single strands should occur as frequently as double strands. Actually, single strands are relatively rare, indicating a considerable amount of non-homologous association. The type of chromosomal association at metaphase I is as follows:

Types of Association -	7_{III}	$6_{III}{}^{+1}{}_{II}{}^{+1}{}_{I}$	$5_{III}{}^{+2}{}_{II}{}^{+2}{}_{I}$	$4_{III}{}^{+3}{}_{II}{}^{+3}{}_{I}$
Number of sporocytes -	59	44	22	2

At anaphase I, of 57 sporocytes examined, 24.7 percent showed one lagging univalent, 14.0 percent showed two, 5.3 percent showed three, and 10.6 percent showed four. Also 8.8 percent showed a dicentric bridge and acentric fragment. Part of the daughter half chromosomes from the anaphase I laggards formed micronuclei and the remainder were apparently included in the microspore nuclei.

Meiotic Behavior in Tetraploid Lolium perenne

The average quadrivalent frequency at metaphase I for three autotetraploid plants of perennial ryegrass (induced by colchicine treatment, see 1938 Annual Report, pages 33-37) was 3.2, 3.3, and 3.8, respectively. This range is similar to that found for naturally occurring autotetraploid grasses (see page 57). The percentage of metaphase I sporocytes showing univalents was 13.25, 16.33, and 17.65 and the maximum number of univalents in any sporocyte was 6, 4, and 2, respectively, for the three plants. The percentage of anaphase I sporocytes with one or more lagging and dividing univalents was 25.90, 41.86, and 18.66 and the percentage of quartets showing one or more micronuclei was 18.94, 37.40, and 33.90, respectively, for the three plants. In these three meiotic irregularities (univalents at metaphase I, lagging univalents at anaphase I, and micronuclei in the quartets), the induced autotetraploids showed greater meiotic instability than the average of the natural autotetraploids. However, one or more of the natural autotetraploid plants exceed the three induced autotetraploids in each irregularity. Data from more plants of autotetraploid Lolium perenne are needed before an exact comparison is possible.

Cytogenetics and Breeding of Legumes

Fertility Studies in Trifolium repens

Genetics of Cross-Incompatibility

Preliminary studies to determine the genetic factors causing cross-incompatibility were carried on during the winter of 1938-39 by making controlled pollinations in the greenhouse using both related and unrelated plants. Some of the plants used in this work were progeny from crosses made the previous winter as outlined in the 1938 Annual Report. Also described in last year's report is the technique of suction emasculation and controlled pollination used in all this work.

The plants in the first series were two parents, together with 13 of their F_1. This cross was chosen because both parents had proven to be highly self-sterile when selfed in the greenhouse in the winter of 1937-38 and when bagged in the field in the summer of 1938. Rather incidentally, one parent has a distinctive solid white color inside the v-marking on every leaflet, a character for which it was desired to obtain plants homozygous. The 13 F_1 plants were selected because of their general vigor and their profuse flowering in the field, since it had been observed the previous winter that those plants which flowered best in the greenhouse were those plants which had flowered well the summer before in their first year's growth in the field. The 13 F_1 were crossed reciprocally in all combinations and were backcrossed reciprocally to both parents. Every combination was made in duplicate, and a few were made three or four times. It was considered that the largest number of seeds obtained most accurately measured the true potentialities of any combination. A lower number appeared to have resulted simply from poor pollination or damage during emasculation and crossing rather than from a reduced compatibility or environmental condition. The duplicates were generally made by different persons, and some were made on the same day, while the interval between others ranged up to seven weeks. In every combination duplicates gave the same results. The exact number of seeds usually varied some between duplicates, but there was no question as to whether a cross was compatible or incompatible. The average of the higher seed set in all compatible combinations was 44.9 per 10 flowers crossed, whereas the average obtained from the 114 incompatible crosses and selfs that were made, including duplicates, was 0.26 seeds. The 13 progeny were found to consist of four intra-sterile, inter-fertile groups of five, four, three, and one plant, respectively, and every plant was reciprocally fertile with both parents. These results seem to be explained best at present by the diploid personate type of multiple oppositional factors where the parents differed in both factors.

A second series of plants was chosen to study the effect of high pseudo-self-fertility on cross-compatibilities. The plant used as original female parent had shown the highest self-fertility of all plants tested at that time, yielding an average of 115 seeds

per head when manipulated under bag in the summer of 1937. Likewise, its yield of selfed seed in the greenhouse during the winter of 1937-38 and in the field during the summer of 1938 was among the best of all plants tested. The other parent had proven to be highly self-sterile in the same three periods of testing. A further discussion of the pseudo-self-fertility of these plants and their progeny will be included below. It will suffice to state here that the factors conditioning this type of self-fertility appeared to be independent of the cross-incompatibility factors, since four intra-sterile, inter-fertile groups of four, four, three, and two, respectively, were found in this second set of 13 F_1 plants. All backcrosses were compatible except those using two progeny groups as females and the original pseudo-self-fertile parent as male. No adequate explanation of these results in terms of a diploid series of oppositional allelomorphs has been postulated at present. The distinction between compatible and incompatible crosses was clear, the former averaging 41.9 seeds per 10 flowers crossed, while the latter averaged 0.18 seeds. Here also practically every cross was made twice and a few were made three times, often at several weeks' intervals between duplicates. In no case did duplicates give different results.

In both series the four genotypes among the progeny are different from those of the parents and together with the two parental genotypes they constitute the six possible combinations of arranging in pairs the four different allelomorphs brought together in each pair of original parents. All reciprocal combinations between the six groups of the first series and the six of the second series were made to test whether any genotypes were the same in the two series. Every combination was compatible except those using the pseudo-self-fertile parent of the second series as male and one parent and two progeny classes of the first series as female. The reciprocals of these three combinations were compatible and can probably eventually be interpreted in the same way as the incompatible backcrosses in the second series. Since no reciprocals failed, at least three of the factors in one series must be different from the four in the other, or, in other words, at least seven different factors in the multiple allelomorphic series have already been established.

Additional evidence was obtained leading to the conclusion that the allelomorphic series conditioning fertility in white clover must contain a large number of different allelomorphs. Four unrelated plants were found to be compatible with all 12 groups from the first and second series, and three of these plants were compatible when intercrossed in all combinations. The latter crosses were part of a series of matings between 14 supposedly unrelated plants. Of the 91 possible combinations, 62 were made (or 96 of the 182 possible crosses including reciprocals), and all were compatible. In addition, over 50 miscellaneous other crosses between unrelated plants were made, and all were compatible.

A corollary observation on these crosses showed that within 24 - 72 hours after pollination any combination could be predicted as being compatible if the standards rolled in and turned brown and the pedicels reflexed. After a corresponding incompatible cross there was no external change in this time.

A demonstration paper describing some of these results was presented at the meetings of the American Association for the Advancement of Science at Columbus (see page 57) and it is expected that the complete results of these experiments, together with any additional data, will be published during 1940.

Cytology of Incompatibility and of Embryo Development

The cytological basis for incompatibility was studied with whole mounts of the stigma and style in lactophenol and with permanent preparations of the pistil. Crosses or selfs for these studies were made by pollinating five flowers with compatible pollen and another five on the same head with incompatible pollen. In the whole mounts at 8 - 20 hours after pollination, when the greatest differences were observed, the rate of germination was much greater, the percentage of germination was much higher, and the speed of producing long pollen tubes was much greater in the compatible pollinations than in the incompatible. For example, typical preparations 16 hours after an incompatible pollination showed that most of the pollen had not germinated and that the few pollen tubes present were very short, whereas after a compatible pollination practically all of the pollen had germinated and most of the grains had produced long tubes. With permanent preparations of the pistil the ultimate behavior of the tubes was studied. Within four hours after a compatible pollination, the longest tubes were seen at the bottom of the stylar canal, within 8 hours at the bottom of the ovary, within 16 - 20 hours fertilization had taken place, and within 24 hours 2- to 4-celled embryos were observed. On the other hand, even 36 and 48 hours after an incompatible pollination only a few tubes were ever observed in the lower end of the style, and none were seen on the placenta. The few seeds resulting from incompatible crosses must have arisen as the result of an exceptional tube growth despite the inhibition on stigma and in style. Such exceptions were probably too infrequent to be observed cytologically. Once an egg has been fertilized after an incompatible pollination the development seemed to proceed normally without abortion and to give rise to normal seeds.

The normal embryo development after compatible pollinations was very rapid. From the 2- to 4-celled structure at 24 hours, the embryo usually proceeded to the 8- or 12-celled stage by 48 hours after pollination and was many celled by 72 hours. The disintegrating synergids and the end of the pollen tube that had apparently been responsible for fertilization, together with its tube nucleus, were often seen as late as 48 or 72 hours after pollination. Five days after pollination differentiation had begun and at 15 days the hypocotyl, epicotyl, and cotyledons were practically morphologically mature. Within 20 - 30 days after pollination seeds were ripe enough for harvest in the greenhouse.

Controlled Pollination with Bee Cages

Eight bee cages were set up in the field last summer to test

(1) whether certain combinations found compatible or incompatible with hand pollination in the greenhouse would give the same results with bee pollination in the field and (2) whether different degrees of self-compatibility that were measured by manipulating under bag in the field would hold true under bee pollination. The cages were 4 x 4 x 8 feet, covered on the sides, end, and top with 12-mesh window screen (see Figure 1, B). This cage size was used in order to cover two spaced plants in the field, but in a few cases where there was a fairly large alley between the two selected plants another whole plant was transplanted in the spring into this space. When the plants had begun to flower, all opened heads were carefully picked off and the cage placed over the plants. On the same day that the cage was thus prepared, two frames of emerging brood to which adults were clinging were put into a four-frame swarm box, together with a queen cell and two frames of foundation. In order to render inviable any pollen the bees carried, the opening in this small hive was screened until the next day, at which time the opening was placed against a small hole cut in the bottom board of the cage and the screen removed so that the bees could fly only inside the cage. The bees were left for 5 - 6 weeks in the cage, and the first harvest of seed was taken off shortly after the bees were removed. During the time that the bees were confined to the cage, a standard sugar syrup was provided from a feeding can through a small hole in the inner cover of the hive. A jar of water was also provided inside of the cage. The old bees spent most of their time flying against the top of the cage, but the young bees began almost at once to visit the flowers in the cage. It was observed that bees in the open rarely visit a head which another bee had recently visited, but in the cage, where there was an excess of bees for the number of flowers, the bees often visited heads that other bees had just left. Good pollination must have been affected as shown by the number of seeds obtained in compatible crosses or in selfing the plant with high self-compatibility. When the heads were threshed, the 25 cleanest and fullest heads were picked out first, and the number of flowers and seeds counted. It was thought that these heads had probably been better pollinated and would give a better measure of the fertility relationships. The total seeds were also counted for all the heads harvested, especially to get an estimate of the total seed producing capacity of plants under cages. Although there were on the average about the same number of flowers per head with sister plants of the first series under cages I, II, and III, under cage II (compatible cross) there was about 80 times and under cage III (compatible cross) about 90 times as many seeds produced as under cage I (incompatible cross). The same relationship held, but not quite to the same degree, with plants of the second series under cages IV and V. These differences are of nearly the same magnitude as those obtained in the greenhouse by manual pollination and are certainly of practical significance. It should also be noted that the bees were able to produce about the same number of seeds on selfing the highly self-fertile plant 37 W 58(160) under cage VII as in the compatible crosses under cages II, III, and IV. Data from the second harvest is not as extensive, but practically the same degrees of differences prevail.

Open-pollinated seed was picked from sister plants of the first and second series and from unrelated plants picked at random in the nursery. In every case the number of seeds per head is larger than with compatible crosses under cages. On the 25 best heads, the number of flowers per head is on the average also larger than the average under the cages, but this difference is not proportionally as large as the seed difference and probably not significant. The larger seed set in the open might be accounted for by (1) different plant material, (2) better pollination in the open, or (3) more favorable environment in the open. The third explanation is the only one to which significance is attached under the circumstances of the experiments.

Self-Fertility

A large number of the plants in the 1938 nursery were bagged last summer, but only part of this material has been threshed to date. Nevertheless, several observations on self-fertility have been made during the year and will be briefly described here.

It was noted above that the original female parent of the second series was the most highly self-fertile of all plants tested in the summer of 1937. When this plant and its F_1 were selfed in the greenhouse last winter both with and without emasculation and even in the bud they were self-sterile to about the same degree as highly self-sterile plants. This type of pseudo-self-fertility seems to be brought into effect only by rubbing the heads to self-pollinate them. This was first shown in the greenhouse last winter. It was even more striking in the field last summer where the F_1 in the second series averaged 10.4 seeds per head with a range in individual averages from 0.9 to 36.1 and the F_1 in the first series averaged only 1.7 seeds per head with a range in individual averages from 0.6 to 3.2. From this it is concluded that this type of high pseudo-self-fertility is definitely a heritable character and the original pseudo-self-fertile parent is probably heterozygous for the genes conditioning the fertility.

Of all the plants tested so far very few have given indications of having true self-fertility. One of the best examples is plant 37 W 11(47). When it was first bagged in the field in 1938, four heads set an average of 143 seeds. In the greenhouse last winter it bore only two heads. One was manipulated as fast as the flowers opened and it set 266 seed; 10 flowers on the other head were selfed without emasculation and they set 34 seeds, a total comparing well with compatible crosses. A few inbred plants have also given indications of true self-fertility under all conditions. The inheritance of this character is now being studied.

Most of the bagging to measure the self-fertility of different plants has been done with only two bags per plant and two heads in each bag. To show how inadequate this is, the data are summarized in Table 3 from a large number of bags that were put on four clones selected for the rust study where it was primarily desired to obtain a large amount of selfed seed.

Table 3.- Results of selfing a large number of heads on four clones of white clover.

Plant No.	Total bags (2 heads per bag)	Range in seeds per bag	Av. seeds per head and S.D.*
37 W 44(11)	38	12 - 74	42.6 21.3
37 W 49(59)	28	1 - 34	16.0 8.1
37 W 49(107)	47	0 - 66	17.9 12.1
37 W 72(173)	13	4 - 73	30.2 18.0

* Standard deviation of a single determination.

This large variability for every plant is probably attributable for the most part to the variations inherent in the manipulation technique. It seems evident that large sampling errors must be introduced when only a few bags are used per plant.

Varietal Improvement in Trifolium repens

Inbreeding Studies

In the spring, 1939, there were set out in the field 77 inbred lines; 60 consisted of 30 plants each, 13 of 60 plants, and 4 of 120 plants. Each of the lines was interplanted with parental clones in three randomized blocks in the same manner as the 1938 inbreeding study outlined in last year's report. In August all of the inbred plants were individually rated in comparison with the parental clones growing adjacent. A plant of approximately the same vigor as the parents (on the basis of spread, leafiness, etc.) was classified as 10, the smallest survivors in the field as 1, and all others on a proportional scale. At the same time the inbreds in the 1938 planting were similarly scored in their second year's growth in the field. In Table 4 are summarized the mean rating of the inbreds in

Table 4.- Vigor rating of inbreds as compared with parents.

Inbreeding Expt.No.	Date when notes taken	No. of parents	Total No. surviving I_1	Mean rating of inbreds		Frequency distribution of I_1 4	5	6	7	8	9	10
1938 No.1	8-24-38	26	690	7.11		1	1	9	5	4	6	
	8-28-39		537	6.21	7.00	1	7	10	6	2		
No.2	8-24-38	12	228	6.68	6.33	1	3	2	3	2		1
	8-28-39		177	6.70		2		4	2	3	1	
1939 No.1	8-28-39	30	850	7.40				4	13	12	1	
No.2	8-28-39	30	863	8.53					4	8	17	1
No.3	8-28-39	13	622	8.39	7.94				3	3	7	
No.4	8-28-39	4	465	7.20					3	1		
Totals and average		115	3718	7.71								
			1st yr. only	1st yr. only								

each of the experiments and their frequency distribution. It is clear that the inbreds started in 1939 received a better average score than the 1938 inbreds in either their first or second year. Several factors may have been responsible in part for this difference. In addition to the two plantings consisting of different plant material and being grown in different years, it should be noted that the field with the 1939 nursery was well fertilized last spring, whereas the other field has not been fertilized for several years. Moreover, the summer of 1939 was much more favorable for white clover growth than the summer of 1938, during which there were several weeks of severe drought. The data also seem to indicate that the second-year growth of inbreds may be poorer than their first year's growth. In general it appears that on the average 20 to 30 percent reduction in vigor may be expected from first generation inbreds of white clover when they are compared directly with clones of their parents.

Combining Ability of Selected Plants

In the summer of 1938 there were selected from the 38 parental clones in the inbreeding experiments the 15 which had produced the most vigorous and uniform progeny. Slips of each were brought into the greenhouse last winter and an attempt was made to obtain all diallel crosses. One plant failed to flower and most of the others flowered sparsely, so that only 62 of the 91 possible combinations (or 96 of the 182 crosses including reciprocals) were made. All seed obtained was germinated last spring and 3363 individuals were set in a fairly uniform area in the field. At no time during the past summer could significant differences in vigor between these progenies be observed in the way that differences have been noted between parents and first generation inbreds. In fact, the plants over this entire area were strikingly uniform in vigor and the only differences observed could apparently be accounted for by soil heterogeneity. This preliminary experiment seems to indicate that a combining ability test will not provide much useful information on breeding-behavior differences if the parents in the test have first been selected because they produce uniform and vigorous inbreds.

Evaluation of Plant Types in Sod Plots

White clover sod plots were established in the summer of 1938 as described in last year's report. This past summer the high-cut plots were mowed five times and the low-cut six times, the latter two cuts in both series being harvested for yield respectively on July 10-11 and August 14, 15, 16. The ranges of yields, measured in total grams of dry matter, are shown in Table 5. The differences between extremes were obviously significant, in every case the highest being four or five times as great as the lowest. These differences were clearly reflected in the quantity and quality (i.e., color, density, etc.) of Kentucky bluegrass growing with the clover. Several general observations on the growth of different morphological types were

Table 5.- Range in grams of dry-matter from two clippings of white clover sod plots.

	High-cut	Low-cut
July	33 - 121-1/4	34-1/2 - 132-3/4
August	10-1/2 - 50	21-1/2 - 104-1/4

made throughout the season. Good sods were usually not formed by bunchy types, by very prostrate plants, and by very open-growing clones. The lower growing plants perform well only under the low-cut, whereas the higher growing plants seem to adapt themselves better to both heights of cutting. Profuse flowering did not seem to be as much an inhibitor of good growth in sod as in spaced plants. The best sod was formed by the taller, more spreading, and more densely growing plants. It was frequently noted, however, that the growth type is not always correlated with a particular type of sod. Apparently the only way to be sure of the sod-forming ability of any plant is actually to test it in sods. A great difference between clones was noted in their ability to recover after clipping, some coming back much faster than others, although they appeared equally good before cutting. It was also noted that the low-cut plots seemed regularly to have less reserves to be used in recovery than the high-cut ones. It should be emphasized that all these observations were on the second-year's growth of the plots, and they may not hold for subsequent behavior. (See Figure 1, A).

Genetical Studies in Trifolium repens

Isolation of Genetic Markers and Preliminary Crossing Results

All inbred lines have been classified wherever possible for those characters which may be easily distinguished in the field, such as leaf-markings, flower-color, etc. Some of the second and third generation inbreds are homozygous for certain of these characters and controlled crosses are being made for further study. Although most inbred progenies have been relatively small for genetic classification, most of the clear cut segregations have approximated a 3:1 ratio. This past summer in two first-generation inbred lines the first segregations for chlorophyll deficiency were noted. One segregated 22 normal:7 dificient and the other 20:7. A few other results are shown in Table 6. The character of white inside the v-marking is carried by plants of the first series. The F_2 plants and backcross progeny are both deficient in the recessive solid green class, but the dificiency may not be significant. The other segregations noted in table 6 are with plants of the combining-ability test. These F_1 results indicate in each case that the parent

Table 6.- Segregations for various "markers"

Segregation observed	Exp't. ratio	X^2	P
152 white inside v-marking:36 solid green	3:1	3.433	.10 - .05
64 white inside v-marking:43 solid green	1:1	4.121	.05 - .02
208 red spot:226 absence of spot	1:1	.747	.50 - .30
192 light green inside v-marking:196 absence of light green	1:1	.0412	.90 - .80
227 red stripe:201 absence of red stripe	1:1	1.579	.30 - .20

carrying the dominant marking was heterozygous, and that the character is probably inherited in a simple diploid manner. The F_1 plants resulting from the crosses between the unifoliolate and normal leaved plants were all found to be normal leaved in the field last summer.

HCN Tests of Inbred Lines and Parents

This past summer 1123 first-generation, 489 second-generation, and 68 third-generation inbreds, as well as clones of their parents, wherever available, were tested for HCN by a modification of the qualitative test of Rogers. Plants were simply graded as follows: 0, no change in color (no HCN); 1, trace of brown color; 2, light brown; 3, medium brown; 4, dark brown. Lines homozygous for large amounts of HCN were found in the first-, second-, and third-generation inbreds. For example, all of the 68 third-generation inbreds were graded 4 except two which had slightly less than the maximum color and were called 3-1/2. There were also some first- and second-generation inbred lines that had no HCN, all the plants testing 0. Some of the first-generation lines were segregating into some plants high and other plants 0. Part of these gave approximately 3:1 ratios of plus and 0 plants, while others showed more nearly 1:1 ratios. The remaining lines gave an indeterminate behavior with a few plants 4 and 0 and the remainder of all intermediate grades. Crosses have been made between high and 0 lines and the segregating generations will be studied especially for the correlation of HCN with vegetative growth and longevity.

Rust Reaction of Inbred Lines and Parents

A genetic investigation of the resistance and susceptibility of white clover to Uromyces trifolii repentis was begun jointly by the pathologistand geneticist. Observations in the nursery for the past two summers indicate that this is one of the most important pathogens on spaced plants. This disease has never been severe during the first year's growth of the plants, but in the second year it is so serious on some plants that they are practically killed. The first-generation inbred plants and their parents in the 1938 nursery were noted last summer under a natural epiphytotic. Eight lines were homozygous resistant, 10 were homozygous susceptible, and the remaining 20

either definitely segregating (63S:15R) or showed some intermediate type of behavior in that most of the plants were classified as 1, 2, or 3, rather than 0 (no rust) or 4(very severe rust). Crosses between selected homozygous resistant and susceptible parents have been made, and the F_1 plants will be intercrossed and backcrossed in the greenhouse this winter for a study of segregation. Preliminary results indicate that F_1 seedlings are susceptible under artificial inoculation in the greenhouse.

Variation in Number of Ovules per Ovary

The average number of seeds set in last winter's compatible crosses was computed for each plant. For example, the male parent in the first series ranged from 39 to 51 and averaged 45.6, and the female parent ranged from 46 to 53 and averaged 50.3. The averages of the F_1 of these two parents ranged from 35.3 to 49.6. Likewise, the male parent in the second series averaged 44.6, the female parent 53.7, and their progeny showed a range in average similar to that in the first series. The parents might be heterozygous for the genes determining this character, and the character may be quantitatively determined by a large number of genes. The plant setting the largest number of seeds in controlled crosses was one used as a female in 5 crosses last winter from which 48, 62, 63, 67, and 70 seeds were obtained. Thus in the few plants examined so far some have averaged only slightly over 30 seeds per 10 flowers, while one averaged over 60. A cytological examination of pistils from plants in this range, both before fertilization and at regular intervals during embryo development, indicated that the main factor influencing this difference was the number of ovules borne in the different plants. In general, with this method of crossing in which only 10 flowers per head were used, every ovule present was fertilized and developed into a normal seed, even though those at both ends of the ovarian cavity were often smaller in the beginning. Consequently the number of seeds set per flower in controlled crosses is seen to be an exact measure of the number of ovules produced.

Although these differences at first appear of practical significance, it should be noted that under open pollination in the field there are usually only about one and rarely more than two seeds set per flower. In other words, when all of the ovules on the head are competing for food, only one or two per pod ripen into seed no matter how many were there in the beginning. A more important practical consideration for increasing the number of seeds set per head would probably be the number of flowers per head.

Interspecific and Intervarietal Hybridization

Last winter, crosses were attempted between 19 different plants of white clover as females and the following other clover species as males: 4n red, alsike, crimson, strawberry, subterranean,

and zigzag. Normally opened flowers were used in eight crosses and flowers in the bud in 38. Only 3 seeds were obtained, but none germinated.

Likewise, the 4n red clover was crossed as a female with white, strawberry, alsike, and zigzag, but no germinable seeds were obtained.

Eighteen heads of white clover were crossed with Ladino with an average seed set of 36. Apparently this cross is fertile.

Cytological Studies

Regularity of Meiosis in Microsporocytes of Trifolium repens

From plants growing in the greenhouse in the spring of 1938 fixations were made of young buds for a study of the regularity of the reduction divisions. The 11 plants used represent 11 seed collections from Maryland, Michigan, New York, Oregon, West Virginia, and New Zealand. These plants had originally been selected from the 1937 nursery of nearly 10,000 individuals as representing a wide diversity of morphological types. One was Ladino clover, and the other ten were typical of the white clover occurring naturally in pastures of the Northeast. All fixations were with modifications of Navashin's fluid, several of which appeared to fix equally well, but for best results it seemed necessary to prefix for about a minute in Carnoy's or a modification of it and to sink the buds in the fluid by exhausting the air from the vial. By running the buds through cedar oil for clearing before embedding, the relatively hard and brittle flowers were rendered comparatively easy to cut. Sections were cut 12 - 15u and stained with Haidenhains haematoxylin. Some smear preparations were made for comparison, but due to the small size of the anthers at the time of meiosis no really satisfactory slides were obtained.

The regularity of pairing in 610 microsporocytes from these 11 plants was noted at diakinesis and in polar and side views of heterotypic metaphase. Since there is a possibility of some chromosomes being torn out by the knife in sectional material, only cells in which the contents appear intact and in which all 32 chromosomes could be counted were recorded. One side view of IM showed clearly 15 bivalents and 2 univalents, the latter being on opposite sides of the plate. Another side view was of questionable interpretation, and three polar views were questionable. In the questionable irregular cases, there was the possibility that the single chromosomes off the plate had simply precociously disjoined after a previous normal pairing. A few clear cases of precocious disjunction were included among the "regular pairing". All evidence pointed to these being the beginning of normal disjunction. It might be noted that all five cases of precocious disjunction recorded for plant 2(25) came from the same anther sac, in which a number of the cells were in advanced anaphase. It is significant that no cases of polyvalent pairing were observed and the only deviations from 16 bivalents were those in which univalents were observed. Even including

the four questionable cases, the percentage of irregular pairings is only 0.8; calculated on basis of the one clear irregularity it is only 0.16. This low frequency of irregularity is rarely found in diploids and less often in tetraploids. The validity of these observations is emphasized when it is noted that 46 percent of the regular figures recorded are either diakinesis or side view of IM, in both of which there is little chance for misinterpretation when all 32 chromosomes are counted.

The equality of disjunction as seen in 909 IA or IIM chromosome groups from the same 11 plants was recorded. Only 3 cells or 0.7 percent of the groups were other than the regular 16 - 16 type. One IIM clearly had 15 chromosomes in one plate and 17 in the other, with possibly a fragment in the cytoplasm free from both plates. Also two cells of IIM in plant 15(31) were irregular; in both cases there were about 32 chromosomes on only one plate in the cell. No observations have been made which would indicate whether such figures arose from a restoration nucleus or from an irregular premeiotic division. Here also the validity of the observations is strengthened by the fact that 34 percent of the equally distributed groups observed were seen in cells in which both groups or plates were counted, thus minimizing the chance for erroneous counts or interpretation. In fact, all of the 110 IA groups in which both groups were counted had a normal 16 - 16 disjunction.

At IIA all 93 chromosome groups counted showed 16 chromosomes which must have resulted from an equal distribution of the IIM. In 67 percent of these counts both groups of the same figure were observed, consequently, in those cases there was little chance for observation error. This high degree of regularity in number of chromosomes for each microspore fits well with the percentage of regularity at IM, IA, and IIM. No divisions in the microspores were observed.

Only a few isolated divisions have been seen in the developing macrogametophyte, but all had about 16 chromosomes, probably indicating the same regularity of meiosis in the macrosporocytes.

The most important conclusion drawn from these observations is that white clover is probably an amphidiploid rather than an autotetraploid, since even an autotetraploid of long evolutionary standing might be expected to have some polyvalent pairing.

A manuscript has been prepared presenting the results in some detail. (See page 57).

Studies with Colchicined Plants

Last spring slips were started from the 32- and 64-chromosome sectors of 16 plants that had been selected from the colchicine treated seedlings. Originally 10 stolons had been isolated from each plant. Various proportions of these from each plant had shown the doubled number of chromosomes in root-tip counts. One

isolation had about 128 chromosomes, and several showed an odd number of 70-90. Wherever possible 3 replicate slips were taken from all survivors of the original 10 slips from each of the sixteen plants, and transplanted to the field. Throughout their first summer it was difficult to distinguish any important differences between the 32- and 64-chromosome pieces of the same plant. In some few cases one or the other type appeared slightly superior, but never were differences distinct enough to be easily observed. The 128-chromosome piece has consistently been stunted.

A few crosses were made in the greenhouse last winter with the better-growing doubled and undoubled isolations. Seed was obtained in most 64 x 64 crosses, but not as abundantly as in 32 x 32. The crosses, 32 x 64, set some seeds, but the reciprocal was sterile in every case. A few seeds were obtained from the odd-chromosome plants. Most of these progenies are being grown now for cytological analysis.

In further colchicine treatments last spring a few 64-chromosome plants of Ladino clover were obtained.

Only one germinable seed has been obtained from the one tetraploid red clover plant that grew normally. This came from crossing it with 2n red. By cytological examination this F_1 has been found to be triploid (21 chromosomes).

Miscellaneous Chromosome Counts

Root-tip chromosomes from a number of additional plants of white clover, including most of those used in the compatibility studies last winter, were counted, and all were found to be 32 except one plant which had some cells with apparently 33. This checks with the studies on regularity of meiosis.

Root-tips of several plants of zigzag clover, including some first-generation inbreds, have been examined cytologically. The most frequently occurring count was 78, but the figures were very difficult to interpret and counts varied widely, even within the same tip.

Several trisomic plants of sweet clover have been found. They arose from a 24-chromosome plant by selfing, crossing with the diploid, or open-pollination in the field. Very distinct morphological types appear to result from the extra chromosomes.

Photoperiodic and Temperature Responses of Some Perennial Grasses and Legumes

Dactylis glomerata, Lolium perenne, Phleum pratense, and Poa spp.

The material included 17 clones of orchard grass, 14 of timothy, 47 of perennial ryegrass, 25 of Kentucky bluegrass, 48 of Canada bluegrass, and several clones each of other Poa species. After several weeks' growth at 10° to 13° in the greenhouse during November and early December the plants were subjected to the following treatments:

I. Four weeks' cold treatment of 6° to -5° in darkness, followed by one of the following light treatments at 18° to 20° greenhouse temperatures-

Natural day (9 to 10 hours)
12-hour day - 75 f.c. supplementary Mazda light
16-hour day - 10 f.c. supplementary Mazda light
16-hour day - 75 f.c. supplementary Mazda light
16-hour day -125 f.c. supplementary Mazda light
16-hour day -240 f.c. supplementary Mazda light
16-hour day - 75 f.c. Mazda and 75 f.c. mercury vapor lamp

II. No cold treatment-

16-hour day - 75 f.c. supplementary Mazda light

Previous work had indicated (see 1938 Annual report) that a day length of 16 hours would induce several of these species to flower and that a cold period at about 0° for 1-1/2 to 3 months previous to the 16-hour day increased the number of plants heading and the number of heads produced. Because of this experience a majority of the plants were given a four-weeks' cold treatment and thereafter a 16-hour day to bring as many of them as possible into heading to facilitate the cytogenetic work. A limited number of plants were subjected to other treatments which had not been proven effective. Because plants of each clone were not represented in each treatment a comparison of the clonal responses of all clones to all treatments cannot be made. However, the following general conclusions may be drawn.

In this experiment none of the ryegrass, orchard grass, timothy, or Canada bluegrass headed under the normal day of 9 to 10 hours. Several of the Kentucky bluegrass plants which had been subjected to the cold treatment did produce short stocky heads and apparently normal flowers. About one-half of the plants given a 12-hour day at 75 f.c. flowered a week later than the same clones given a 16-hour day.

The intensity of the supplementary light used to make a 16-hour day affected the flowering habits of the plants only slightly between the limits of 75 and 250 f.c. The plants receiving 250 f.c. flowered a day or two earlier but were taller

and more etiolated, (probably due to the heat from the lamps) than those under 75 f.c. The plants receiving 10 f.c. flowered about one week later but only about one-third of them flowered.

The addition of 75 f.c. of illumination for 2 hours daily from a mercury arc to the 75 f.c. of Mazda light under a 16-hour day was apparently without effect.

Several clones of Kentucky bluegrass, timothy, and orchard grass were subjected to supplementary illumination of 60 f.c. from daylight fluorescent lamps and of 100 f.c. from sodium vapor lamps to make a 16-hour day. In both treatments the plants headed and flowered about two weeks later than the same clones under Mazda illumination but the growth was much stockier and darker green in color.

Of 16 other species of Poa, almost all the plants headed when given a cold treatment and a 16-hour day with 75 f.c. supplementary Mazda light of which only a very limited number headed under the natural day length of 9 to 10 hours.

The results obtained from all species used indicate that with the exception of timothy and Canada bluegrass, cold treatments previous to increased day length aids materially in inducing heading.

It is evident that all plants within a species cannot be classed as identical in their photoperiodic response. Some grass clones flowered under a 12- and 16-hour day, while other clones flowered only under the 16-hour day. (See Figure 2, C). Clones which flowered under the shorter day length also flowered when subjected to the longer day length.

Trifolium repens

The eight clones of white clover used in this experiment were selected on the basis of their previous flowering behavior in the field and greenhouse. They included three profusely flowering, two medium flowering, and three sparsely flowering clones. Clonal replicates were started from cuttings in soil in 4-inch clay pots and allowed to become well established in a cool (15° to 18°) greenhouse during November and December 1938. They were then subjected to the following temperature and light treatments:

I. Cold treatment of four weeks at 7° to -5°, followed by one of the following light treatments at 18° to 20° greenhouse temperature-

Normal day
12-hour day - 75 f.c. supplementary Mazda light
16-hour day - 75 f.c. supplementary Mazda light
18-hour day - 10 f.c. supplementary Mazda light
18-hour day - 75 f.c. supplementary Mazda light
18-hour day - 275 f.c. supplementary Mazda light

II. No cold treatment-

Normal day
12-hour day - 75 f.c. supplementary Mazda light
16-hour day - 75 f.c. supplementary Mazda light
18-hour day - 75 f.c. supplementary Mazda light
18-hour day - 275 f.c. supplementary Mazda light
16-hour day - 75 f.c. supplementary Mazda light and 75 f.c. from mercury vapor lamps
16-hour day - 75 f.c. supplementary daylight fluorescent light
18-hour day - 100 f.c. supplementary sodium vapor light

Records were taken on the date of flowering and number of heads produced. These data indicate wide variations between clones of white clover in their response to flowering under various day lengths. Without a cold treatment two clones flowered under the normal day of 9 to 10 hours, another flowered under the 12-hour day, two more flowered under a 16-hour day, one under an 18-hour day, and one did not flower under any of the day lengths used. When a clone flowered under a shorter day length, it also flowered under the longer day length.

The data further indicate that unfrozen plants under an 18-hour day produced almost as many heads when 75 as when 275 f.c. were used. Those plants under 275 f.c. flowered several days earlier but those under 75 f.c. bloomed for a longer period. In the frozen series under an 18-hour day the 10 f.c. of supplementary Mazda light produced as many heads as the two higher intensities of 75 and 275 f.c.

It is apparent from the data that the cold treatment did not aid in inducing flowering except under a normal day. Three frozen plants produced more heads than the unfrozen plants of the same clone. In almost every other case the frozen plants produced fewer heads than those not frozen when given supplementary light to increase the day length.

The plants given supplementary light from sodium vapor and daylight fluorescent lamps were stockier, darker green in color and produced as many flowers as those receiving supplementary Mazda light. The ultra violet radiation from the mercury lamps showed no effect on heading.

Poa pratensis Seedlings

The material used in this study consisted of 600 three-month-old seedlings of Kentucky bluegrass. These were divided into groups, each of which was subject to one of the following cold treatments:

+1° for 7 days
+1° for 14 days
+1° for 28 days
+1° for 56 days
-2° for 7 days
-2° for 14 days

-2° for 28 days
-2° for 56 days
-2° for 7 days; -5° to -9°, 7 days
-2° for 7 days; -5° to -9°, 21 days
-2° for 7 days; -5° to -9°, 49 days
No cold treatment

After the cold treatment random samples from the groups were placed under three light treatments which included (1) normal day length, (2) normal day length supplemented by 75 f.c. Mazda illumination to make a 16-hour day, and (3) normal day length supplemented by 100 f.c. of sodium vapor illumination to make a 17-hour day. The flowering dates and the number of heads produced per pot were recorded.

The results of this experiment indicate that a storage period of 28 days at +1° was most satisfactory for inducing head formation.

A 16-hour day with 75 f.c. from Mazda lamps produced the largest percentage of plants heading and nearly as great a number of heads per plant as the 17-hour day treatment using 100 f.c. of illumination from a sodium vapor lamp.

The lower storage temperature of -2° was slightly less effective than +1°. Storage at -7° was definitely inferior to +1°, presumably due to injury by dessication.

The Effect of Clipping and Nitrogen Fertility on Top, Root, and Rhizome Growth and Water Utilization of Four Clones of Poa pratensis

Clonal isolations were used from four plants of widely divergent morphological characters, namely, narrow leaf, erect; wide leaf, erect; narrow leaf, decumbent; and wide leaf, decumbent. In the first experiment the plants were grown in gravel cultures supplied individually with nutrient using the method described previously (See 1938 Annual Report). The plants were allowed two months to establish themselves on a balanced uniform nutrient solution. One-half of the cultures were then supplied with a nutrient high in nitrogen (50 p.p.m. N) and the other half were supplied with a nutrient low in nitrogen (5 p.p.m. N). All cultures were clipped uniformly to one-inch and the following treatments begun in triplicate:

cut every 10 days to 1 inch
cut every 3 weeks to 1 inch
cut every 4 weeks to 1 inch
cut at 12 weeks to 1 inch
cut when 5 inches high back to 3 inches
cut when 5 inches high back to 1 inch
cut when 4 inches high back to 1 inch
cut when 3 inches high back to 1 inch

The various clones, fertilizer, and clipping treatments were arranged at random over four greenhouse benches. The series which ran from December 8, 1938, to March 2, 1939, were given 100 f.c. of supplementary Mazda light to make a 16-hour day and the greenhouse temperature was maintained at 18° to 22°. A duplicate series carried on from June 24 to September 14, 1939, was under natural day length of 15.5 hours to 12.3 hours with day temperature ranging from 30° to 35°. During this period the greenhouse was shaded, reducing the light intensity by one-third.

In addition to the gravel cultures during the summer, the same clones were grown in soil cultures and one-half of the clipping and fertility treatments repeated.

At the end of the twelve weeks' clipping period all cultures were cut to a 1-inch height and allowed to recover for four weeks (the growth of the plant serving as a measure of its food reserves). (See Figure 2, B). The plants were then clipped at 1-inch and the dry weight of tops, stubble, roots, and rhizomes determined.

In the gravel cultures the greatest differences in response to the above clipping treatments occurred on the high nitrogen nutrient between the narrow-leaved erect clone and the broad-leaved decumbent one. Under a clipping treatment simulating a hay stage (clipped at the end of 12 weeks) the former produced nearly one-fourth more dry top growth than the latter. When other clipping treatments were applied, the relationship was reversed. The more severe clipping treatments reduced the yield of the narrow-leaved erect plants much more than they did the yield of the broad-leaved decumbent plants. When the top growth was removed to one inch every 10 days the broad-leaved decumbent produced over twice as much total dry top growth as the narrow-leaved erect form. The broad-leaved erect and narrow-leaved decumbent clones maintained more or less intermediate positions with respect to their responses to the clipping treatments. In general, clipping every 10 days to one inch appeared to depress the yield of total dry top growth about the same as clipping to one inch when the top growth had reached the 2- and 4-inch heights. Yields of total dry top growth from the cultures clipped to one inch every three weeks approximated those which were clipped to 3 inches when a height of 5 inches had been reached.

When the cultures were supplied with low nitrogen nutrient the amount of top growth was severely limited so that all clones yielded approximately the same. The dry weight of tops produced under the most favorable clipping treatment (at 12 weeks only) was only about twice as great as that produced under the most severe treatment (clipped every 10 days.)

The relative production of fibrous roots by the different clones when supplied with ample nitrogen was similar to the top-growth under the same clipping treatment but showed even greater differences. The narrow-leaved erect plant produced twelve times as much dry root growth when unclipped for 12 weeks as when clipped every 10 days. While this clone produced about the same amount of roots as the broad-leaved decumbent clone, under the

10-day cutting treatment the latter produced three times as much root growth as the narrow-leaved erect plant. Again, the other two clones were intermediate in response.

The root response of the clones supplied with two concentrations of nitrogen was opposite to the top growth response. Under every clipping treatment, with the exception of the cultures clipped at 12 weeks only, the low nitrogen cultures produced more roots than the high. In the case of the broad-leaved decumbent clone under a 10-day clipping treatment, the low nitrogen cultures produced nearly three times as much root growth as the high nitrogen culture.

The production of rhizomes was affected more by the clipping treatments than were either tops or roots. The broad-leaved erect plant produced few rhizomes under the 10-day clipping treatment but when clipped only at the end of 12 weeks, produced an abundance of rhizomes - nearly 70 times the amount produced under the 10-day clipping treatments. The broad-leaved decumbent plant nearly equalled the broad-leaved erect plant in rhizome production when clipped at 12 weeks, whereas when clipped every 10 days the broad-leaved decumbent produced about three times that of the broad-leaved erect plant.

The narrow-leaved erect plant produced only a small amount of rhizome growth even under the most favorable conditions (clipped at 12 weeks only), while under the other treatments it produced no rhizomes whatever or such a small amount that they were negligible.

The low nitrogen nutrient reduced the magnitude of the differences in rhizome formation due to the clipping treatments, but the effect of close or frequent clipping still remained evident.

A comparison of the gravel and soil cultures in the second experiment shows that the latter produced less top growth and the detrimental effect of frequent clipping was greater. Under the high nitrogen fertility and frequent clipping (every 10 days) most of the plants in the soil cultures were dead after eight weeks.

In the gravel cultures the water required per gram of dry matter produced under high nitrogen was roughly one-half that of the cultures under low nitrogen. Water was most efficiently used by the plants receiving high nitrogen and clipped every four weeks. It was used least efficiently by plants clipped only at 12 weeks, and by those severely clipped. Under practically all clipping treatments and with high nitrogen nutrient the broad-leaved decumbent plant was most efficient in water utilization.

Preliminary analyses of total nitrogen of the top growth from the various clones indicate that differences of 20 percent or more exist between clones and that differences of 30 percent exist between clipping treatments of the same clone.

Improvement in Method of Determining Hydrocyanic Acid

In previous work on the hydrocyanic acid content of Trifolium repens it was found that about 70 percent of the individual plants contained .001 percent or less of HCN on a fresh weight basis. In studying individual plants only a small amount of plant material is usually available and microchemical methods of analysis are required.

The method previously used involved a visual comparison of the unknown samples with known standards. An improvement in accuracy has been made by introducing into the method a comparison in the KWSZ photometer.

HCN is now determined by digesting 10 grams of fresh material for several days at room temperature with toluene. The HCN is then carried by steam distillation into KOH solution after which it is heated with alkaline picrate solution. The change in color of the picrate solution is measured by the photometer, using a 10 percent copper sulfate solution as a light filter, and calculations as to the content of HCN are made from a curve previously and permanently prepared. The method which has been published (see page 57) appears suitable for white clover and sudan grass.

Effect of Nitrogen on the Hydrocyanic Acid Content of Sorghum vulgare var. sudanense

Plants from five long-time inbred lines of sudan grass were grown in gravel cultures on nutrients containing 2, 10, and 50 p.p.m. of nitrogen. Samples of leaves were first taken for HCN analysis when the plants had reached the late boot stage. A statistical analysis of the data indicated no significant difference between the nitrogen level supplied in the nutrient and HCN content, but significant differences were obtained in the amount of HCN produced by the various strains. Two subsequent analyses were made of the HCN in the aftermath (when the young growth was 8 to 10 inches high). A significant difference was found in the HCN content of the various strains. Furthermore, the plants which received 50 p.p.m. of nitrogen were higher in HCN than those receiving 10 p.p.m. and these in turn were higher than those receiving 2 p.p.m.

Improvement in Methods of Determining Sugars

The method of determining glucose and fructose in mixture has been further improved.

Following the oxidation of glucose by iodine in alkaline solution it has been necessary to make a correction, often approximate, for the effect of the iodide ion upon the reducing power of the fructose. This correction is now avoided by the quantitative removal of the iodide with lead acetate.

In the oxidation of the glucose, some non-sugar reducing substances are also oxidized by the iodine and appear in the results

as glucose. If, in the aliquot of the plant extract to be used for total sugar determination, iodine is allowed to react in a neutral medium, the sugars are not affected but certain non-sugar substances are oxidized and hence they do not enter into the calculations of either glucose or fructose. These substances oxidized in the neutral medium may not be identical with those destroyed in the alkaline medium. Thus, the use of iodine in neutral solution may materially reduce, if not entirely eliminate, the error due to non-sugar substances. The procedure in use has been modified to include this step.

In clearing plant extracts for sugar analysis it is assumed, because of the presence of non-sugar reducing substances, that the clearing procedure which gives the lowest results for total reducing sugar and which does not affect the sugars themselves, is more nearly correct. The addition of iodine in neutral solution therefore appears to improve the accuracy of the results in some plants as shown in Table 7.

Table 7.- A comparison of the reducing sugar content of plant extracts obtained after clearing with lead and with iodine followed by lead. Expressed as mg. of cu for equal aliquots.

	Cleared with lead only	Cleared with iodine and lead
Sudan grass	4.78	4.72
Apple leaves	3.83	2.74
Peach leaves	7.85	7.54

Polysaccharides of Poa compressa

Further studies of Canada bluegrass have been made with the object of identifying and describing those carbohydrate fractions important as reserves. Equal parts of alcohol extracted tops were subjected to various hydrolytic agents for various periods of time. The data plotted in Figure 2, A, show the amount of glucose and fructors* liberated under various conditions, expressed as mg. of copper per .025 gm. of residue.

To summarize the data there are at least three polysaccharide fractions from which sugars are produced. Fraction I is defined as that which is completely hydrolyzed by 0.25 percent oxalic acid in .75 hour at 80°; it contains approximately 90 percent of fructose and 10 percent of glucose, the amounts of which are not increased by longer hydrolysis. This fraction is also hydrolyzed by 0.5 N sulfuric acid in 0.3 hour; longer hydrolysis does not increase the yield of fructose but does increase the yield of glucose, apparently derived from a more resistant carbohydrate fraction. Boiling

* The terms glucose and fructose have been used, but it should be understood that these substances have not been positively identified.

0.5 N sulfuric acid quickly yields the same amount of fructose, but this is gradually destroyed; considerable amounts of glucose are liberated as well.

Fraction II is defined as that which contains only glucose and is liberated by takadiastase at pH 4.6 and which reaches an endpoint of hydrolysis after 5 days at 38°. No fructose is liberated by takadiastase.

Fraction III contains only glucose and is that which is liberated by boiling acid in excess of that in Fractions I and II. Its properties have not been further defined.

Fraction I is only partly soluble in cold water but is completely soluble in water at 80°, in which Fractions II and III are insoluble. Only a trace of glucose and no fructose is liberated from the plant material by saliva. Invertase has no action on it.

The relative amounts of Fractions I and II occurring in plants grown in soil in the greenhouse are given in Table 8. Fraction I contains relatively less fructose when the amount of that fraction is low.

Table 8.- The carbohydrate content of Canada bluegrass with respect to Fractions I and II

	Fraction I	Fructose in Fraction I	Fraction II
	percent dry wt.	percent	percent dry wt.
First cutting-			
tops	0.85	47.0	7.96
rhizomes	17.47	92.4	7.56
roots	2.80	71.8	3.10
Second cutting-			
tops	7.25	91.4	9.87
rhizomes and roots	33.30	96.0	6.05

Analyses of these fractions were also made on plants grown in sand cultures under 13 different environmental conditions of nutrient, temperature, length of day, and light intensity. Fraction I varied from 1.11 to 8.76 percent of the dry weight in the tops and from 2.27 to 6.55 in the underground parts. Fraction II varied from 5.19 to 9.33 percent in the tops and from 7.05 to 11.05 percent in the underground parts. Fraction I showed the greatest concentration under low nitrogen nutrition. These preliminary observations indicate that Fraction I may be most important as a reserve substance.

Further work on Poa compressa and on Lolium perenne is in progress.

Variation in Crude Protein of Poa pratensis

Further data concerning the range in variation of crude protein in Kentucky bluegrass were obtained. The plants studied were

clonal isolations from pasture throughout the Region and had been started in the nursery in the spring of 1938. The date of emergence of the panicle and the date of flowering were observed for each plant in 1939 and all were mowed on July 8. Aftermath was collected for analysis on August 30, 1939. The percentage of total nitrogen in 164 plants had a range of from 2.7 to 4.2, with a mean of 3.5. Observations of the previous year on other plants of the same species that the later flowering plants are higher in crude protein than the earlier during a nonreproductive stage of growth were substantiated. The coefficients of correlation between the percentage of nitrogen and the dates of emergence ($r = 0.37$) and between the percentages of nitrogen and the dates of flowering ($r = 0.44$) were highly significant. Some data are given in Table 9.

Table 9.- Relation of the total nitrogen content of aftermath of *Poa pratensis* harvested August 30 to the date of flowering.

No. of plants	Date of flowering	Percent total nitrogen
1	May 22	3.22
1	May 23	2.94
1	May 26	3.07
30	May 27	3.33 ± .06
18	May 28	3.36 ± .03
58	May 29	3.48 ± .04
19	May 30	3.66 ± .07
19	May 31	3.70 ± .06
14	June 1	3.65 ± .07
3	June 3	3.76 ± .21

Six clones which had widely different composition in the field in 1938 were grown in sand cultures in the greenhouse under four levels of nutrition. No significant differences were found between clones either in yield or composition when compared with their composition in the field. Considerable, but not uniform, differences were found in the yield of short, medium, and long leaves between clones. There were no significant differences in the percent protein content of different sized leaves.

Variations in Yields and Mineral Content Among Clones of *Poa pratensis*

A preliminary trial in the greenhouse with 80 clones of Kentucky bluegrass indicated marked differences in mineral content and in yield response to phosphorus and nitrogen. On the basis of this trial 18 clones, representing extremes in yield response to nitrogen and phosphorus as well as wide differences in mineral content, were selected for further study. These 18 clones are now growing in quadruplicate in 1-gallon pots filled with Dekalb silt loam at two phosphorus levels and at three nitrogen levels. Yields will be recorded and certain selected samples analyzed for nitrogen and mineral content.

Clonal Variation in Calcium, Phosphorus, and Potassium Content in Trifolium repens

In a preliminary trial, thirty-nine clones of white clover were grown in the greenhouse in 1-gallon pots of uniformly treated soil. When well established the plants were plucked to simulate grazing and samples kept for chemical analysis. The samples were collected in early June at which time most of the clones were flowering. A measure of the amount of flowering was obtained by harvesting the flower stalks separately from the vegetative parts of the plants. Yields of both flowers and leaves were recorded but only the leafes (leaflets plus petioles) were analyzed. The variations in the calcium, phosphorus, and potassium content of these samples are shown in Table 10. The ranges in mineral

Table 10.- Calcium, phosphorus, and potassium content of different clones of white clover grown on the same soil* in the greenhouse.

Frequency classes with respect to percentage calcium					
1.50%	1.70%	1.90%	2.10%	2.30%	2.50%
1	4	9	11	7	7

Frequency classes with respect to percentage phosphorus					
0.165%	0.195%	0.225%	0.255%	0.285%	0.315%
3	12	16	6	1	1

Frequency classes with respect to percentage potassium					
1.05%	1.35%	1.65%	1.95%	2.25%	2.55%
2	15	16	4	2	0

* A Dekalb silt loam treated with PKL, but still deficient in available phosphorus as measured by plant response.

content were calcium, 1.56 to 2.60 percent; phosphorus, 0.149 to 0.321 percent; and potassium, 1.07 to 2.39 percent. These differences were not associated with the degree of flowering. The variations in calcium content appeared to be independent of the yield and of the phosphorus and potassium content. There was a very marked inverse relationship, however, between the yield of dry matter and the percentage phosphorus content.

A few clones that differed widely in mineral content in the preliminary trial were then grown at widely different levels of soil acidity and fertility. The results show that if a clone is relatively high in percentage calcium or phosphorus at one level of soil fertility or acidity, it is also relatively high at all other levels of acidity and fertility. Similarly, clones relatively low in percentage calcium or phosphorus at one soil treatment are low at other soil treatments. Clones 15 (31) and 33 (37) represent the extremes in calcium and phosphorus content. Regardless of the lime and phosphorus levels in the soil, clone 15 (31) produced about twice the yield of dry matter and three times the yield of calcium, but only slightly more phosphorus than 33 (37). It is apparent therefore that the Ca/P ratio of clone 15 (31) is about three times as high as that of 33 (37).

Studies are also being conducted to determine if relative differences in mineral content of various clones are maintained when the plants are grown on widely different soil types, and if differences in calcium and phosphorus content are associated with differences in yield response to calcium and phosphorus.

Chemical Composition of Diploid and Tetraploid Lolium perenne

Following treatment of seeds of perennial ryegrass with colchicine, diploid and tetraploid clones were obtained from certain seedlings by vegetative reproduction (see 1938 Annual Report, page 33). Cuttings were grown in ten rows in the greenhouse bed. Each of five original seedlings were represented by two rows distributed at random and each row contained eight individual cuttings consisting of three to five diploid and three to five tetraploid individuals. At the end of two months the plants were still in a vegetative condition and were harvested on February 3, 1939. The tops of all diploid plants in one row were composited and the tops of all tetraploids in the same row were also composited in another sample. Ten such pairs of samples were obtained.

Analyses were made for total dry matter, for the proportion of dry matter soluble in 80 percent alcohol, for crude fiber, reducing sugars, sucrose, alcohol soluble nitrogen and alcohol insoluble nitrogen.

The only statistically significant differences noted between diploid and tetraploid clones were in the higher content of sugars and alcohol soluble dry matter in the tetraploid samples. (Table 11.) Yield data and other constituents studied gave insig-

Table 11.- Comparative analysis of diploid and tetraploid Lolium perenne. Mean of 10 samples in percentage of dry weight.

	Diploid	Tetraploid	Significance t*
Alcohol soluble dry matter	31.94	32.73	2.64
Reducing sugars	.94	1.17	2.86
Sucrose	3.09	3.49	3.66
Total sugars as invert	4.18	4.84	4.14

* Values of t for P of 0.05 and P of 0.01 are 2.262 and 3.250, respectively.

nificant differences. These data have been published (see page 57).

An experiment is under way in which diploid and tetraploid clones in gravel culture are being compared as to their yield, composition, and water utilization under various nutrient levels and clipping treatments.

Preliminary studies to Evaluate Drought Resistance

The term drought resistance as used in this section refers to ability to produce relatively good growth during a dry period

as well as the capacity to endure wilting.

Growth at Low Soil Moisture

A preliminary investigation was made in the greenhouse to determine if relative drought resistance could be evaluated by supplying the same amount of water to each pot and determining the yield of dry matter. Six species, Poa pratensis, Trifolium repens, Dactylis glomerata, Lolium perenne, Agropyron smithii, and Festuca rubra, each represented by a single clone, were used for this trial. Red fescue and western wheatgrass were selected as supposedly drought resistant species; white clover and perennial ryegrass were selected to represent the less drought resistant species; and orchard grass was selected because of its reputation for a deep root system. Each species was grown in duplicate under each of the following conditions:

1. In 1-gallon glazed pots
2. In 1-gallon glazed pots but with less water than No. 1
3. In boxes 18 inches square and 12 inches deep
4. In boxes 8 inches square and 48 inches deep

The deep boxes were filled to a depth of 36 inches with Hagerstown clay subsoil. The top 10 inches was a soil-sand-manure mixture. The other containers were all filled with the soil-sand-manure mixture. Liberal quantities of a complete fertilizer were applied several times during the experiment. The surface of the soil was covered with 1/2 inch of fine gravel to decrease evaporation.

The plants were started in the pots and boxes in the greenhouse in the fall. On March 3, when they were well established, they were given a uniform clipping treatment. Each container was then saturated with water and any excess water allowed to drain from a hole at the bottom of the container. Thereafter, the 1-gallon pots and the shallow boxes were watered at irregular intervals in an attempt to simulate roughly the conditions that might occur during a dry summer. In the deep boxes the soil was saturated twice more during the experiment, once on June 24 and again on August 3. The sixth and final clipping was made September 13. The deep boxes were then dismantled and the root system examined.

The total yields of dry matter are shown in Table 12. There is a good agreement between the yields obtained in the shallow boxes and in the 1-gallon pots, but these yields do not agree with the reputed drought resistance of the species. Western wheatgrass and red fescue, supposedly drought resistant species, produced less dry matter than any other species, whereas perennial ryegrass was second highest. The total yields, however, do not tell the whole story. Perennial ryegrass produced more during March than did any other species, but did not hold up as well during the summer as did Kentucky bluegrass and orchard grass. Kentucky bluegrass was particularly outstanding during May, June, and July. White clover made poor growth from the middle of June until the con-

Table 12.- Yields of various plant species under artificial drought in relation to the type of container.

Species	Total dry matter (grams per pot or box)				Relative yields				
	Shallow boxes	1-gal. pots	1-gal. pots (very low water)	Deep boxes	Shallow boxes	1-gal. pots	1-gal. pots (very low water)	Av. for pots & shallow boxes	Deep boxes
Poa pratensis	120	24.4	17.8	37.6	100	100	100	100	100
Trifolium repens	91	19.1	15.0	35.2	76	78	84	79	94
Dactylis glomerata	110	21.0	15.5	47.1	92	86	87	88	125
Lolium perenne	120	20.4	16.7	39.6	100	84	94	93	105
Agropyron smithii	87	17.3	13.7	41.5	73	71	77	74	110
Festuca rubra	75	18.1	12.9	15.9	63	74	72	70	42

clusion of the trial, whereas western wheatgrass made relatively better growth during this period than during late winter and spring.

In the deep boxes the relative yields were quite different from those in the shallow containers. Orchard grass produced 197 percent more than red fescue, whereas in the shallow containers it averaged only 26 percent more. Similarly, western wheatgrass was 162 percent better than red fescue in the deep boxes but only 7 percent better in the shallow containers. The striking differences between the performance in the deep containers and in the shallow ones can be explained on the basis of the differences in root systems. In the shallow container all of the added water except that lost by evaporation was available for the plants. Since evaporation was small in comparison with transpiration, each plant received approximately the same amount of water. In the deep boxes, however, it was noted that after the boxes had been saturated red fescue was always the first plant to show the effect of insufficient water and would cease growth, while the bottom of the box was still saturated with water. Orchard grass and western wheatgrass on the other hand would readily remove the water from the bottom of the box. Kentucky bluegrass and perennial ryegrass was considerably less efficient than orchard grass and western wheatgrass in this respect, but superior to white clover. These observations were substantiated by the examination of the root system in the boxes at the conclusion of the experiment. (Table 13).

These preliminary results suggest that this type of investigation may have a place in the evaluation of pasture plants.

Table 13.- Root distribution and soil moisture in the deep boxes at the conclusion of the experiment.

Species	Condition of plants	Soil moisture	Root distribution
Agropyron smithii	nearly dead	entire soil column dry	Good root development throughout the entire soil mass
Dactylis glomerata	nearly dead	entire soil column dry	Slightly less roots in the bottom of the box than under Agropyron
Poa pratensis	green and growing slowly	soil moist at bottom of box	Poor root system in subsoil compared with Agropyron and Dactylis, but a few roots have penetrated nearly to the bottom
Lolium perenne	nearly dead	Soil at about the moisture equivalent near the bottom of the box	About comparable to Poa
Trifolium repens	green and growing slowly	Considerably more moisture in the bottom of the box than under Lolium	No roots within 8 - 10 inches of the bottom of the box
Festuca rubra	nearly dead	Only the 10-inch layer of surface soil was dry. Even the top few inches of the clay was at approximately field capacity	Very few roots in the subsoil

Capacity to Endure Wilting

Preliminary trials with Trifolium repens and Poa pratensis growing in association in a pot of soil, showed that if water were withheld white clover would be killed before the grass was seriously injured. As a result of further trials with other species a clone of Lolium perenne and one of Phleum pratense have been selected that are injured to about the same extent as the clover growing in association with them. Using these selected clones of grass as standards it appears that some clones of white clover are better able to withstand this artificial drought than are others. Whether or not these differences are associated with drought resistance in the field remains to be seen.

Germination of Freshly Harvested Seeds of Dactylis glomerata and Poa spp.

Freshly harvested seed of Poa pratensis will germinate promptly after the moistened seed is subjected to a temperature of about 7° for 10 days (see 1938 Annual Report). Further studies were conducted on the temperature and storage time necessary to obtain good germination

from freshly harvested dormant seeds of several Poa species and Dactylis glomerata. Results from these studies indicate that daily alternating temperatures from 10° to 30° or 15° to 30° are slightly more effective in inducing germination in dormant seeds of Poa pratensis than a continuous temperature of 10° or 15° for 10 to 14 days followed by alternating temperatures from 20° to 30°.

Storage of moistened freshly harvested seed of Poa compressa and Poa palustris at 10° for 10 days greatly increased germination. Freshly harvested seed of Poa alpina and Poa arachnifera did not germinate at all with alternating temperatures of 20° to 30°, but following 10 days of storage of the moistened seed at 10° the former germinated 100 percent and the latter 24 percent.

Freshly harvested field seed of Dactylis glomerata germinated only 23 percent with alternating temperatures of 22° to 28°. When the moistened seed was subjected to alternating temperatures of 10° to 28° and 15° to 28° the germination increased to approximately 93 percent. Similar results were obtained with continuous temperatures of 10° and 15° for 10 days followed by alternating temperatures of 22° to 28°.

Preliminary experiments indicate that freshly harvested dormant seed of Trifolium repens may also be induced to germinate with low temperatures.

Germination of Sorghum vulgare var. sudanense at Various Temperatures

In a preliminary study of optimum temperatures required, six lots of open-pollinated sudan grass seed from Illinois, Ohio, and Nebraska were germinated on blotters in petri dishes at temperatures of 5, 10, 15, 20, 25, 30, 35, and 40 degrees. The germination of all lots indicated that temperatures between 20° and 35° were most favorable and that 10°, 15°, and 40° were less favorable. Alternating temperatures of 20° to 30° did not increase the percent germination. At 5° there was no germination in 18 days.

Pathology

The phytopathological investigations at the Laboratory during the year were concerned primarily with Trifolium repens and Sorghum vulgare var. sudanense, although some investigations were made of the flora of seed of Trifolium pratense. Some attention was given also to identifying and determining the extent of various pathogens occurring on pasture plants.

Field Trips

Approximately 20 days were spent on field trips. Pastures, meadows, and experimental plots of experiment stations were visited in Pennsylvania, New Jersey, Delaware, Maryland, New York,

Massachusetts, and Connecticut. Material was collected for isolation and observations made concerning prevalence and importance of diseases.

A fungus similar to the "Black patch" fungus of Valleau was isolated from one plant of red clover near State College, Pennsylvania. This is probably the first time this fungus has been found in Pennsylvania.

One pasture was seen in Pennsylvania severely infested with Helminthosporium vagans. The fungus was found in nearly all pastures examined.

Diseases Found in the Plant Nursery at State College, Pa.

White clover was severely attacked again this year by rust. Parent plants, together with their first generation inbred progeny, were found which were very resistant under this natural epiphytotic. Cercospora zebrina, Pseudoplea trifolii, Polythrincium trifolii, and Stagnospora spp. were prevalent. Colletotrichum destructivum was found but did not seem to be as plentiful as in 1938. Winterkilling was severe in some white clover plants.

The red clover plants present in the plots were severely injured by mosaic, nearly all being infected. Mildew was heavy on some plants.

Approximately five percent of the Lotus corniculata plants in the nursery were killed by a fungus, presumably Sclerotinia trifoliorum.

Sudan grass was heavily attacked by Helminthosporium turcicum and Colletotrichum graminicola, and bacterial lesions occurred.

Scolecotrichum graminis attacked various species of grasses, and was especially severe on Canada bluegrass again this year.

Orchard grass, timothy, and perennial ryegrass were severely attacked by rust. Probably resistant plants were found in orchard grass and perennial ryegrass. Possible resistance occurred in a few plants of timothy.

Life History and Variation of Colletotrichum destructivum

This fungus was isolated from diseased plants collected in Pennsylvania, New York, Massachusetts, and New Jersey. Inoculation experiments with single spore cultures demonstrated pathogenicity. It is probably a relatively weak parasite. The host range to date includes, Trifolium repens, T. pratense, T. hybridum, Medicago sativa, M. lupulina, Melilotus alba, and M. indica. Comparisons of single spore cultures of Colletotrichum destructivum and C. trifolii were made and it was found that C. destructivum grew more vigorously,

possessed longer and narrower spores, and had a somewhat wider temperature range than C. trifolii. It is probably a valid species.

A study of the possible cause of loss of sporulating ability in culture of Colletotrichum destructivum was continued from 1938. Conidia were reported previously to be primarily uni-nucleate and further cytological work confirms this, in no case more than two nuclei being found. Poorly sporulating variants, and variants which sporulated more profusely than the original cultures, were obtained. As many as fifteen distinct cultural types arose from one single spore culture (Figure 3, A), indicating heterocaryosis was not responsible for the variation.

Seed Treatment of Legumes

Preliminary studies of the effect of seed treatment on stands of five species of legumes were begun. The species were red clover, alfalfa, yellow sweetclover, white sweetclover, and Korean lespedeza. The seed disinfectants used were 5 percent ethyl mercury phosphate and cuprous oxide. Results to date indicate treatment to be beneficial, the cuprous oxide being more efficacious in minimizing post emergence damping off, while the 5 percent ethyl mercury phosphate was better with respect to reducing pre-emergence damping off (Figure 3, C).

The Flora of Trifolium pratense Seed

Seed from thirty-three lots produced in eight states (Colorado, Idaho, Illinois, Michigan, Montana, Ohio, Tennessee, and Virginia) were surface sterilized and plated on potato dextrose agar. Eighteen were found to be infected with Pleospora herbarum, five with Macrosporium sarcinaeforme, one with Cercospora zebrina, and two with the "black patch" fungus of Valleau and Johnson. A species of Phoma occurred in four seed lots. Various saprophytic species were isolated also.

Occurrence of Helminthosporium turcicum in Sudan Grass Seed

This fungus was isolated from the seed or glumes of 21 of 52 seed lots produced in ten States, tested by surface sterilization and planting on potato dextrose agar. As high as 20 percent of the seed and 50 percent of the glumes were infected in some lots. The fungus was found in the seed and glumes of seed lots two winters old (Figure 3, B). Germination was found to be somewhat lower in tests made in the laboratory and field with various infected seed lots as compared with non-infected lots. Besides saprophytic fungi, Colletotrichum graminicola was isolated from several seed lots.

A manuscript has been prepared summarizing the results (see page 57).

Seed Treatment of Sudan Grass

One year's results in the field with 29 lots of sudan grass seed gave statistically significant results (above one percent point), indicating increased stand when seed were treated with 2 percent ethyl mercury chloride over controls. The stands of check and treated were 50.4 percent and 57.0 percent, respectively.

Pythium Injury to Sudan Grass

A pythiaceous fungus has been isolated from sudan grass seedlings, which, in sterilized soil, has reduced stand from 94 percent in checks to 8 percent in inoculated soil (Figure 3, D). Surviving plants were severely stunted. Its importance and prevalence is unknown.

Life History Studies of Scolecotrichum graminis

This fungus was found in a viable condition on old material of Canada bluegrass in pastures during the winter. Lesions on green leaves were also present. Cultural studies continued from 1938 indicate a multiplicity of distinct cultural races. Preliminary temperature studies indicate the minimum to be below 5°C., the optimum between 20° and 25°, and the maximum between 30° and 35°. Experiments to induce sporulation in culture were unsuccessful.

SUMMARY

Cooperative activity-

Two important meetings were held during the year, the plant breeding conference in June and the collaborators' meeting in October. As a more or less direct outgrowth of these meetings four cooperative projects were developed between certain State stations and the Laboratory. Three of these projects are concerned with breeding and one with pathology of pasture plants. Preliminary plans are under way to develop additional cooperative projects.

During the annual collaborators' meeting, attention was focused on the pasture research programs carried on at the State Agricultural Experiment Stations, rather than the program at the Laboratory, as was done in previous years. Proposed new State projects were discussed. Since this meeting a conference involving two State stations and the Division of Forage Crops and Diseases was held to consider a proposed pasture experiment at Montrose, Pennsylvania. Tentative State projects have been circulated among the collaborators for their criticism and suggestions.

Cytogenetics and breeding of grasses-

Treatment of heads of timothy with water at 46° and 48° apparently effected complete inactivation of the pollen without damaging the ovules. Self-fertility in orchard grass, perennial ryegrass, and timothy, as measured by seed set under parchment

bags, appear to be conditioned by multiple factors. Great variation in seed set was found between bags on the same plant, between plants and between years. First inbred generation families of orchard grass, perennial ryegrass, and timothy are reduced in vigor but all are sufficiently vigorous to permit further inbreeding. Second generation inbreds have been established in orchard grass and perennial ryegrass. Progenies of individual Kentucky bluegrass plants varied from those in which all plants were similar to the parent to those in which more than 50 percent of the plants differed from the parent. Approximately 400 families of sudan grass, inbred from two to four generations, have been established.

Crosses of male sterile with male fertile plants have been made in orchard grass, perennial ryegrass, and timothy. Diallel crosses of heterozygous plants of orchard grass and timothy have been planted for use in studies of heterosis and combining ability. Further observational data and some yields were taken on the sod plots established from single clones in the spring of 1938. The plots were clipped periodically throughout the growing season.

Parental clones and inbred progenies of orchard grass, perennial ryegrass, and timothy were classified for reaction to natural rust epiphytotics. Considerable resistance was found in orchard grass and perennial ryegrass. Several differential characters for use as genetics markers have been found in perennial ryegrass. Tetrasomic inheritance has been found to occur in orchard grass.

Induced autotetraploids in perennial ryegrass have broader, thicker, and longer leaves; thicker culms; larger spikes, flowers, and seeds; fewer tillers; and somewhat lower fertility than diploids. No differences were found in date of maturity or chloroplast size. Stomatal size was only slightly greater in the tetraploids. The chromosomal association and behavior have been studied in three natural autotetraploid species, orchard grass, tall oat grass, and crested wheatgrass. Wide differences in meiotic irregularities were found between plants. Only 59 percent of the orchard grass plants which were studied had the euploid chromosome number of 28. All plants studied in the three species were heterozygous for one or more inversions. Variations in amount of different types of meiotic irregularities were found in 19 diploid plants of perennial ryegrass. A triploid plant of perennial ryegrass was obtained and its chromosomal behavior studied. Plants of induced autotetraploid perennial ryegrass were found to resembel naturally occurring autotetraploids in meiotic behavior.

Cytogenetics and breeding of legumes-

Cross-incompatibility in white clover seems to be controlled by a series of multiple allelomorphs. Seven genes in the series have already been established and some evidence has been obtained which indicates that the series may be extensive. Genes outside of this series appear to condition the high pseudo-self-fertility of certain plants. Poor pollen germination and pollen-tube growth apparently accounted for failure to set seed in the incompatible crosses. Embryo development was very rapid after compatible crosses, ripe seed being produced in 15 to 20 days. Compatible and incompatible crosses made by the "suction technique" in the

greenhouse also proved to be compatible and incompatible when made by bee pollination under cages in the field. Likewise, similar differences in self-fertility were obtained in the field by manipulation under a muslin bag and by bee pollination. The manipulation technique used in the field study of self-fertility gave results subject to a large experimental error. A few plants have been found which seem to be highly self-fertile under all conditions. This true self-fertility is apparently conditioned by factors different from those which condition pseudo-self-fertility. A range has been found in average number of ovules per ovary from slightly over three to more than six.

When compared directly with their respective parental clones, first generation inbred families were on the average from 20 to 30 percent less vigorous. A somewhat greater difference in vigor was observed between parental clones and inbreds during the second season than during the first. A combining ability test between plants selected because they produced uniform and vigorous inbreds gave uniformly vigorous first generation plants. In the second season's growth of the clonally established white clover sod plots it was noted that, in general, the taller more spreading and more densely growing plants formed the best sod when associated with Kentucky bluegrass. Yields of the sod plots showed great variation due partly to the difference in aggressiveness and vigor of the various clover clones.

Several genetic characters have been found among inbred families of white clover. In these families and in controlled crosses clear cut ratios approximating 3:1 and 1:1 have been obtained. First, second, and third generation inbred lines have been found homozygous for relatively high and no HCN. Lines homozygous resistant and homozygous susceptible to Uromyces trifolii repentis have been discovered. Interspecific crosses have yielded no germinable seed, but the intervarietal cross, White X Ladino, has been made with ease.

Meiosis has been observed to be regular in 11 plants of white clover, only 0.16 percent of the cells having other than 16 bivalents at diakinesis or first metaphase and only 0.7 percent of first anaphase or second metaphase cells showing other than the normal 16 - 16 disjunction. It is concluded that white clover is probably an amphidiploid, rather than an autotetraploid. Root tips of several plants of zigzag clover have been examined for chromosome number. The counts were variable but the most frequent occurring one was 78. Several trisomic plants of sweet clover have been found among which distinct morphological types appear. The 32- and 64-chromosome sectors from the same plant of white clover did not show any important differences throughout their first summer in the field. The sectors were obtained by colchicine treatment in the seedling stage.

Physiology and composition of pasture plants-

Plants of orchard grass, perennial ryegrass, timothy, Kentucky bluegrass, and 16 other Poa species were induced to head and flower in the greenhouse during the winter months by subjecting them to temperatures from 6° to -5° for four weeks, then supplementing normal day length with artificial light to make a 16-hour day. There was little difference in heading response due to the

intensity of supplementary light between 75 and 250 f.c. All plants within a species were not identical in the length of day required to bring them into head. Three-months old seedling plants of Kentucky bluegrass were induced to head by a cold treatment for four weeks at +1° followed by a 16-hour day in the greenhouse. Germination of freshly harvested dormant seed of Poa species and orchard grass was greatly increased by constant low temperatures of 10° and 15° for 10 days followed by alternating temperatures of 20° and 28°.

The method of determining hydrocyanic acid in white clover has been improved in accuracy by incorporating into the method a photometric comparison. In the determination of reducing sugars in plants some of the effect of non-sugar reducing substances may be avoided by oxidizing these with iodine in neutral solution. The polysaccharides of Canada bluegrass have been grouped into three fractions; one containing about 90 percent fructose is readily hydrolyzed by oxalic acid; a second containing only glucose is hydrolyzed by takadiastase; a third containing only glucose is more resistant to hydrolysis. The first appears to be most important as reserve substance.

When Kentucky bluegrass plants are mowed following flowering, the aftermath of the late flowering plants was higher in crude protein than the early plants. Tetraploid plants of perennial ryegrass were higher in sugars and soluble dry matter than diploid plants of the same genic base. Clones of white clover that differed widely in calcium and phosphorus content when grown on an unproductive Dekalb soil maintained their relative differences in composition where varying quantities of lime and phosphate were applied to the soil. Preliminary trials with 80 clones of Kentucky bluegrass indicated marked differences in yield response to nitrogen and phosphorus.

Preliminary trials with six clones, representing six species, showed that when moisture was a limiting factor, the yields in deep boxes were proportional to the depth of the root system. Preliminary studies were conducted to determine the variation in the capacity of different clones of white clover to endure wilting when the soil was allowed to dry slowly. Four clones of Kentucky bluegrass were found to vary in their ability to withstand severe clipping treatments. Frequent clipping limited rhizome growth to a greater extent than either top or root growth. A low nitrogen supply reduced the effect of clipping. The severity of the clipping treatments affected the water utilization of the plants. One clone was found to be more efficient in the use of water than the other clones.

Pathology-

Field trips were made to determine importance and prevalence of various diseases of pasture plants. Observations in the nursery indicated that orchard grass, perennial ryegrass, white clover, and possibly timothy plants resistant to rust occurred. Various pathogenic fungi were isolated from the seed of sudan grass and red clover. Preliminary results of the effect of seed treatment on stands of various clovers and sudan grass indicate a beneficial effect. Colletotrichum destructivum was studied in comparison with Colletotrichum trifolii and is probably a valid species. The reason for loss of sporulation in culture of C. destructivum was studied. Some cultural studies of Scolecotrichum graminis were begun. A pythiaceous fungus has been isolated from sudan grass seedlings and demonstrated as pathogenic.

LIST OF PUBLICATIONS

Published-

Garber, R. J. The Agronomist, His Profession, and an Example of Coordinated Research. Jour. Am. Soc. Agron. Vol. 31, No. 12. Dec. 1939.

Myers, W. M. Colchicine Induced Tetraploidy in Perennial Ryegrass. Jour. Hered. 30: 499-504. 1939.

Sullivan, J.T. Determination of Hydrocyanic Acid by the Picric Acid Method and the KWSZ Photometer. Jour. Assoc. Off. Agr. Chem. 22: 781-784. 1939.

Sullivan, J.T. Report on Carbohydrates in Plants. Jour. Assoc. Off. Agr. Chem. 22: 480-481. 1939 (1938 Report).

Sullivan, J.T., and W. M. Myers. Chemical Composition of Diploid Lolium perenne. Jour. Am. Soc. Agron. Vol. 31, No. 10: 869-871. 1939.

Papers presented-

Atwood, S. S. Cytogenetics of Incompatibility in Trifolium repens. Presented before Genetics Society of America, Columbus, Ohio, December 28-30, 1939.

Myers, W. M. Tetrasomic Inheritance in Dactylis glomerata. Presented before Genetics Society of America, Columbus, Ohio, December 28-30, 1939.

Myers, W. M., and Helen D. Hill. The Association and Behavior of Chromosomes in Autotetraploid Grasses. Presented before Genetics Society of America, Columbus, Ohio, December 28-30, 1939.

Sullivan, J.T. Report on Carbohydrates in Plants. Presented before Association of Official Agricultural Chemists, Washington, D. C. October 31, 1939.

Manuscripts pending-

Atwood, S. S. Regularity of Meiosis in Microsporocytes of Trifolium repens.

Chilton, S. J. P. Occurrence of Helminthosporium turcicum in the Seed and Glumes of Sudan Grass.

Myers, W. M., and Helen D. Hill. The Association and Behavior of Chromosomes and the Occurrence of Aneuploidy in Autotetraploid Grass Species, Orchard Grass, Tall Oat Grass, and Crested wheatgrass.

Myers, W. M. Preliminary Experiments on Self-Fertility in Orchard Grass, Dactylis glomerata.

Myers, W. M. Variations in Chromosomal Behavior in Plants from Normal Populations of Perennial Ryegrass, Lolium perenne.

(The responsibility for compiling each State report was assumed by the collaborator of that State).

Appendix A

PROGRESS REPORT OF STATE STATIONS

Connecticut (Storrs) Agricultural Experiment Station

Project No. 12 - Alfalfa Experiments-

a. Response to soil treatments.- On soils with reactions of pH 6.0 or above and with 30 or more pounds of easily soluble P by the Truog method, practically all of the beneficial effects of stable manure appear to be due to the K it supplies. The effects of manure at 20 tons were mostly obtained in the first two years after application.

b. Effects of time and frequency of cutting alfalfa under different treatments.- Harvesting the third crop October 15 was much less injurious to the stand and subsequent yields than cutting September 20. The former had greater amounts of carbohydrates in the roots. Very large applications of potash are now being used to learn if this nutrient will reduce some of the harmful effects of September and immature cuttings.

c. Effects of amount and depth of applying limestone.- On acid soil (pH 4.8) quite satisfactory stands and yields have been obtained from using limestone at only one ton per acre provided the limestone was spread on the surface or only mixed with the upper two inches of soil. When three tons were used, the placement in the plow layer caused no appreciable differences in the behavior of the alfalfa.

d. The role of minor elements in fertilizing alfalfa.- In combination with several fertilizer treatments, borax at 20 pounds per acre (2.3 pounds boron), applied in August, 1938, was very definitely beneficial to alfalfa on field plots during the dry summer of 1939. It prevented the yellowing of the top leaves and the withering of the flower buds before blossoming. Stable manure at 10 tons per acre in 1936 and again in 1938 reduced somewhat the prevalence of the boron deficiency symptoms. Alfalfa on plots heavily fertilized with potash from 1915-1923 showed the symptoms less than that on adjacent plots, which received no potash between 1915 and 1923.

Mn, Cu, and Zn have so far failed to benefit alfalfa on the Charlton fine sandy loam of the experimental fields at Storrs.

Project No. 14 - The Maintenance and Improvement of Pastures-

a. The effects of fertilizer treatments on the soil, the flora, and the production as measured by grazing.- This project, now in its nineteenth season, is being conducted in about the same manner as in previous years.

Limestone applied on the surface in 1924 and repeated in 1929 had affected appreciably the pH of the sixth inch of soil in 1938. At that time, superphosphate (16 percent) at 500 pounds in 1924, 1929, 1932, and 1935 had increased the easily soluble phosphorus slightly in the third inch.

Without any tillage or seeding, the variations caused in the prevalance of some important species by different fertilizer treatments are now (September 1939) as follows:

White clover - 1 to 13 percent of area
Bluegrass - 1 to 73 percent of area
Bentgrass -10 to 48 percent of area
Weeds - 4 to 39 percent of area

Over a six-year period, 1932-1937, the production, as measured by grazing with dairy heifers, was nearly doubled by superphosphate alone, more than doubled by superphosphate and limestone, and tripled by mineral and nitrogenous fertilizers. No amounts or time of application of nitrogen, tried so far, has raised the level of production from July through October to that obtained in May and June.

c. The effects of various chemicals on the soil, on the botanical composition of the sward, and on the stands and growth of Kentucky bluegrass and Rhode Island bent grass.-

Sodium nitrate has affected the pH of the surface inch of soil less, but the second and third inches more, than any of the other carriers of nitrogen. This result is evidently due to the mobility of the Na ion, for when either NaCl or Na_2SO_4 was applied, the surface inch became more, while the lower layers became less, acid. Na_2CO_3 has decreased markedly the acidity of the first three inches, while K_2CO_3 has had little effect on this factor. Both, but particularly the latter, increased the potash in the grasses.

With an original soil pH of 5.5, NH_4Cl or $(NH_4)_2SO_4$ to supply 84 pounds of nitrogen per acre annually, increased the acidity so much in two years that the stands of Kentucky bluegrass became thinner and the prevalence of weeds, especially sorrel, much greater. This was not true in the case of bent grass. Kentucky bluegrass responded much more than Rhode Island bent grass to very heavy applications (168 pounds per acre) of nitrogen from a neutral carrier.

d. The adaptability of varieties and species of grasses and clovers for pastures.-

After two years of lawnmowing (when 4 inches to 1 inch), Kent County (England) ryegrass had the best stand (43 percent) of five strains; S50 timothy had the highest (70 percent) of seven strains; Tardus grazing strain of orchard grass with 65 percent topped eight others; while none of three strains of meadow fescue had over 37 percent stand. In general, strains developed under grazing conditions maintained better stands than the others.

Ryegrass has produced very little during the summer months, when there is usually shortage of pasturage.

In numerous tests, Rhode Island Bent grass has outyielded Kentucky bluegrass. Ladino clover, alone or in mixtures, continues to yield more than any other variety or strain of white clover. Also, it was affected less by dry weather.

e. Relation between physical and chemical characteristics of the soil and the response to fertilization of vegetation on permanent pastures.- No new data was obtained on this project during the past year.

f. Causes of fluctuations in the prevalence of white clover.-

Sources of seed, fertilization, kinds of accompanying grasses and closeness of cutting or grazing have affected the longevity of white clover.

During the wet summer of 1938, native clover increased on most of the grazed plots, while marked decreases occurred during the dry summer of 1939. The more prevalent the clover, the greater the relative reduction due to drought.

h. Effect of subsoil on response of pasture species to fertilization.- No new data was obtained on this project during the past year.

Project No. 28 - Physical and Chemical Studies of Soils-

Limestone applied to the plow layer at from 7 to 10 tons between 1914 and 1919 had affected the pH of the 30-36 inch horizon in 1937. Eight years after surface application of limestone to grassland with an original pH of 5.5, the pH had been affected significantly to depths of from 3 inches by one ton to 18 inches by sixteen tons. In one year, surface applied limestone at 0.5 ton reduced the acidity of the soil in lawn plots by 0.43 and 0.11 pH in the first and second inches of soil, respectively.

Grass Silage.- During the winter of 1938-39, the Dairy Department conducted a feeding experiment, involving three groups, each with three Jersey cows and one Guernsey heifer, fed for one hundred days as follows:

Group 1 - Alfalfa grass silage as the sole source of roughage (consumed an average of 63 pounds per head daily).

Group 2 - Thirty pounds of the alfalfa silage per head daily plus all of the second cutting alfalfa hay they would consume.

Group 3 - Thirty pounds of corn silage per head daily plus hay as for group 2.

Notes: (1) The alfalfa silage was made from the first cutting and was about 60 percent alfalfa and 40 percent mixed grasses. Sixty pounds of molasses were added to each ton of green material.

(2) Grain was fed according to milk production but its content was changed to keep animals at the same weight.

(3) Average daily milk production:

Group 1 - 38.0 pounds
Group 2 - 37.7 pounds
Group 3 - 39.5 pounds

New Project.-

Title: Response of Alfalfa to Application of Plant Nutrients to Subsoil.(This is a sub-project of 12-a "Response of Alfalfa to Soil Treatments).

Object: Some farmers, who have grown alfalfa for fifteen-twenty years have reported failures of the crop to maintain stands for over two years. This project has been planned to learn if this deep-rooted species reduces the supply of easily available minerals of the subsoil to a point where it seriously affects it longevity.

Procedure: To date, all that has been planned is a very limited experiment involving six plats, each four feet square, and treated only with carriers of Ca, Mg, P, and K, as follows:

1. LPK* mixed with 0-6"
2. LPK mixed with 0-6" plus removing and replacing 6-12" and 12-18" layers
3. Same as (2) plus 1/2 (LPK) in 6-12" layer
4. Same as (3) plus 1/2 (LPK) in 12-18" layer
5. LPK mixed with 0-6" plus removing and replacing 6-12", 12-18", 18-24", and 24-30" layers
6. Same as (5) plus 1/4 (LPK) in each of 6-12", 12-18", 18-24", and 24-30" layers.

* L = dolomitic limestone at 6000 pounds per acre
P = 40 percent superphosphate at 200 pounds per acre
K = 60 percent muriate of potash at 200 pounds per acre

These plats are to be about six feet apart and located on a field on which alfalfa was seeded in 1926. That alfalfa was gradually replaced by grasses until in 1931, very little alfalfa was present. The area was plowed in the spring of 1939 and fallowed to date.

Samples will be taken of each removed layer of soil.

This experiment may be expanded to include minor as well as major elements, changing the placement of only one element at a time, other soil types, and other methods of applying the fertilizers.

Delaware Agricultural Experiment Station

Lespedeza selection for pasture types-

The work with lespedeza was started at the Delaware station in 1934. Some 3400 selections were made, principally from different sources of the Korean variety (Lespedeza stipulaceae). Many strains showing differences were, of course, found. The most promising ones have been retained. Among the factors considered in the elimination of undesirable strains were height and vigor of plants, ability to survive adverse conditions, resistance to disease, ability to set seed, etc. At the present time a few strains are beginning to stand out as very promising, particularly from the standpoint of amount of forage produced. The point has just been reached where it is possible to test these selections as to their ability to compete with the common pasture plants and to survive under actual pasture conditions.

Maine Agricultural Experiment Station

Project: Bankhead-Jones 1.

Title: Pasture and Hayland Improvement.

Leaders: D. S. Fink and F. Chadwick, Jr.

a. Farm permanent pasture fertility tests.- Completed. Reported in Maine Agricultural Experiment Station annual report for 1937-38.

b. Field pasture trials at station farm.- At Highmoor Farm there are twenty-one pasture paddocks from which yield measurements are being obtained in terms of milk produced per acre. These pasture paddocks include several pasture crops differently fertilized and managed. An original check paddock (native permanent pasture) is being left unfertilized to serve as a bench mark for measuring such items as soil and crop improvement. Preliminary reports on yields obtained from several of the paddocks may be found in the Station Annual Report.

Two new paddocks have been established this past season.

(1) A paddock seeded down to a mix of Ladino clover and smooth brome grass. It is proposed to determine the suitability of this crop association when used only for pasture.

(2) A paddock was planted to zigzag clover (3 feet in row and 6 feet between rows) and clean cultivated the entire season. It is proposed to subdivide the paddock into fourths this spring and seed down after first cultivation to four standard pasture grasses. The paddock to be further subdivided into fourths, crosswise of the grass seedings, and four differential fertilizer treatments given. The paddock will be grazed as often as appears desirable. The best plant association, palatability, and treatment will be estimated through observation.

Considerable work is being done with Ladino clover at the Station farm. It has been definitely established that not more than two pounds of Ladino clover seed per acre is necessary to give very satisfactory stands in Maine. A pure stand of Ladino clover has given the largest milk yield per acre to date (5,125 pounds 4 percent milk).

One new project is proposed in connection with Ladino clover; that being, to determine the effect of a side-delivery vs. dump rake on Ladino clover sward when the crop is harvested as hay. A six-acre field was established for this purpose this past season.

c. Alfalfa nursery.- In cooperation with the U. S. Department of Agriculture, Bureau of Plant Industry, an alfalfa variety test is being conducted at Highmoor Farm. Mr. Russell Bailey is in charge of this nursery.

d. Laboratory investigations.- Soil samples from the differently fertilized pasture paddocks and hay fields are being analyzed periodically for changes in chemical characteristics. The initial low

level of soil fertility for several of the pasture paddocks has been raised to an optimum level, as indicated by milk yields and observed through soil analyses, and attention will now be focused on soil fertility maintenance requirements.

e. Grass silage.- An experiment dealing with grass silage is proposed for the comming season, the object of the experiment being to determine the feeding value of molasses added as a preservative over and above that necessary for adequate preservation. The silage will be made from Ladino clover, practically pure stand, in two forty-ton lots; each lot made separately in two, new 10 x 30 foot wood silos. One lot will contain 30 pounds molasses per ton of green material, and the other 120 pounds. The feeding value of each lot will be measured by way of milch cows and yearlings.

f. Manure conservation.- A detailed laboratory investigation is in progress dealing with the conservation of nitrogen in farm manure, especially as it relates to the use of fermented manure as a top-dressing for pasture.

Maryland Agricultural Experiment Station

Development of work in breeding pasture plants has been delayed during the year due to the fact that the land where this work was conducted has been lost and new land did not become available until fall.

Comparisons have been continued between local and commercial strains of pasture plants including Kentucky bluegrass, white clover, orchard grass, and perennial ryegrass. The Kentucky bluegrasses, both local and commercial vary so much from one strain to another that it is difficult to make any general statements concerning the results of these comparisons. However, some of the most promising local strains appear to make slightly greater summer growth than is made by the best strains from elsewhere. Kentish wild white clover is noticeably less tolerant of hot dry summer weather than the local strains of wild white. In fact, local strains either small or of Ladino size appear to be superior in growth and duration to the corresponding commercial strains with which they have been compared.

The most striking superiority of local material over commercial stocks appears in the perennial ryegrasses. Foreign strains (Paceys, Kentish, and Svalof) scarcely survive the summer at all, while local strains contain plants sufficiently resistant to the diseases of summer and fall so that it should be possible to isolate promising stocks for local use. Preliminary observations on seedlings from local plants resistant to rust indicate that resistance is inherited to a large degree.

Seed lots of local orchard grass selections and of both standard and Ladino type local white clovers, together with commercial stocks, will be planted this spring for comparisons on new pasture land.

A graduate student, A. O. Kuhn, completed a study on the effect of removing different proportions of foliage on strains of Kentucky bluegrass and of perennial ryegrass. A brief account of the findings

with respect to Kentucky bluegrass appear in the Journal of the American Society of Agronomy for October 1939.

In this study two contrasting strains of Kentucky bluegrass plants grown in pots were clipped at two-week intervals so as to remove equal proportions of the foliage from each strain. One was a tall- and the other a low-growing strain. With each strain increased severity of clipping resulted in equally increased injury to growth of roots, of rhizomes, and of tops. The tall-growing strain when clipped at about two inches in height, was as severely injured and had about the same proportion of its foliage removed as the low-growing strain when clipped at one inch in height. The perennial ryegrass strain was less severely injured by equal severity of clipping than either of the Kentucky bluegrass strains with which it was compared. Possibly an important reason for the reduced injury to the perennial ryegrass was the quickness with which new growth appeared after clipping. As a consequence of this quick recovery the average severity of defoliation was reduced quickly after each defoliation.

Massachusetts Agricultural Experiment Station

In Massachusetts there are four principal factors which govern pasture production. They are in the order of their importance as follows: 1. Soil fertility. 2. Grazing management technic. 3. Species and species strains. 4. Climate.

High quality pasture herbage is probably the most profitable crop which can be produced in Massachusetts, but this is true only when the cultural requirements of this crop are given the same consideration which is now given to other valuable crops grown in the State. Failure to recognize this fact in the past is largely responsible for the present very poor condition of our pastures. It must be recognized that before desirable pasture species can produce large quantities of palatable, nutritious herbage, the soil must be able to supply them with adequate quantities of all plant food nutrients. The general level of soil fertility must be relatively high. Although the practices which may be necessary to maintain a high level of soil fertility in pasture sods may differ from those followed with other crops and they may also differ from one section to another, the principle of maintaining an adequate level of soil fertility as a prerequisite to satisfactory herbage production, applies universally.

As pastures become more productive as a result of improved soil fertility relationships and the presence of heavier-producing species, grazing management technic becomes increasingly important. Such practices as rotational grazing, the omission of early spring and late fall grazing, the clipping of undergrazed areas and the spreading of droppings are all practices which are not only beneficial but in many cases essential to the maintenance of high productivity levels.

By directly exercising a considerable degree of control over the first three factors which govern pasture production, the adverse effects of the fourth factor, climate, are greatly reduced.

Permanent pastures.- Pasture areas which have never, or only infrequently, been tilled and seeded are included in this category. Ten years' experience with fertilizer topdressing experiments on permanent pasture sods in Massachusetts has shown that, although the composition of the vegetation can be greatly improved and a marked increase in production effected, it is not possible in the great majority of cases to develop and maintain a first-rate pasture by the use of topdressing materials alone. The explanation may be found by examining existing natural soil fertility relationships. The natural level of soil fertility in most Massachusetts pastures is very low, and topdressing applications of fertilizers alone do not raise this level sufficiently high to encourage the establishment and growth of the more productive, desirable pasture species.

Semi-permanent pastures.- This includes those plowable areas which are tilled, fertilized and seeded to a pasture mixture once every 3 to 6 years. Pastures of this type have given very satisfactory results in Massachusetts and the acreage of such pastures is increasing rapidly. The success of semi-permanent pastures may be largely explained on the following basis:

1. Tillage of the soil, accompanied by adequate fertilization improves the soil's physical condition and raises the level of soil fertility to such a point that nutritious and productive pasture species are able to establish themselves and remain productive for several years.

2. The use of a seeding mixture has permitted the introduction of desirable, high yielding pasture species which not only increase the total production of pasture herbage but also aid in maintaining a more uniform production of herbage throughout the pasture season.

3. The importance of desirable grazing management practices has been frequently demonstrated on pastures of this type with the result that grazing management technic, in general, is given careful consideration. This favors amore uniform production of a high quality herbage threughout the grazing season and aids in prolonging the life of the seeding.

The Improvement of perennial pasture plants.-

Strain introduction nursery.- Observations made during the past season on several hundred strains of grass and legume species indicate that the following strains have particular merit:

Species and strain	Seed Source
Italian ryegrass	
Hinderupgaard	Denmark
E. F. 486	Denmark
English ryegrass	
Sutton's Indigenous	England
O. A. C. #1	Canada
E. F. 79	Denmark

Timothy	
Aberstwyth S48	England
Otofte	Denmark
Hankkejan	Finland
Bartimoty	Sweden
F. C. 3937	Ohio, U. S. A.
F. C. 19505	Ohio, U. S. A.
Meadow fescue	
Karlström	Finland
Red clover	
Sutton's Late Flowering	England
Otofte I	Denmark
Tystofte I	Denmark
Hinderupgaard I	Denmark
Early Otofte I	Denmark

New Hampshire Agricultural Experiment Station

Project No. 3 - A Study of Pasture Species Under New Hampshire Conditions-

During the spring of 1939 about 2500 individual plants were grown spaced in flats in the greenhouse and later transplanted to a nursery on the University Farm. These plants represent fifteen species and strains, although by far the major portion of them consist of but three species, viz. timothy, red clover, and wild white clover.

Our first objective in this work is to isolate certain promising, native strains of these three species, selected for the following purposes:

a. Red clover
   For perennial habits
b. Timothy
   Suitability for pasture purposes
   Later maturity than normal
c. Wild white clover
   To develop a pure strain of native stock, that is high yielding and persistent.

Seeds of red clover and timothy plants were chosen from pastures, roadsides, and other waste places during the autumn of 1938. Cuttings of native white clover plants were made in the spring of 1939. Seeds and cuttings were propagated in the greenhouse early in 1939, and later transplanted as previously described.

In addition to the native wild white clover cuttings, some material was also taken from Ladino and New Zealand wild white clover that had been grown in plots in our strain trials during 1936-1938 inclusive, with the idea that these strains might be used as breeding material later on when suitable selections can be made.

Selections of native red clover made in 1936 and seeded in spaced rows in the nursery in 1937 have now gone through their third season.

With respect to the native timothy selections made in 1938, about 10 percent of the plants grown from seeds appear to conform to either the hay-pasture or the true pasture types, kinds first isolated by the Welsh plant breeding station and a few of the plants appear to belong to the diploid or true pasture strain, although we have not as yet verified this by microscopic analysis.

In addition to this individual plant propagation, we seeded a about 60 small plots in a pasture during the spring of 1939, the seeds consisting of many of the "improved" grasses and clovers that have been developed in other parts of the world.

In this test simple mixtures were used consisting of only one grass and one legume. Strains from Wales, Scotland, Sweden, and Canada, as well as from various points in the United States were used. The plots were seeded in duplicate and botanical analyses will be made of them from time to time to note longevity, persistence, ability to withstand competition, and suitability for New Hampshire conditions.

In the 1938 report we published yield records of plots seeded in 1936 to various pure lines and mixtures and harvested at intervals during the 1938 season. The plots in question were left in situ until the spring of 1939 when stand counts were made to determine how the various species had survived during the three-year period, and to note as well the encroachment which any native species had made into the stands of the various plots.

The data from these counts are rather bulky and will not be presented at this time, but certain rather obvious deductions may be made from them which may have some bearing on recommendations that are to be made in the future with respect to pasture seedings.

1. Ladino clover survived this three-year test better than any other white clover pasture in the test, occupying 47.5 percent of the stand in plots in which it was seeded alone at the end of the three-year period.

Plots which had been seeded to mixtures containing Ladino carried 26 percent of this clover at the end of the three-year test. Plots within this group that had smooth brome or orchard grass as components of the mixture carried considerably more Ladino than those in which Reed canary grass had been used. This was doubtless due to the greater competition offered by the Reed canary grass.

2. Kentucky bluegrass, although seeded in but four of the 64 plots, appeared in all plots except four at the end of the period, averaging slightly over 30 percent of the stand in those plots in which it appeared.

This appears to indicate that the ultimate pasture on this soil and in this climate and under close clipping or grazing will be composed largely of Kentucky bluegrass, or with such Dutch or wild white clover as are able to compete with it.

3. The survival of Reed canary and orchard grass was somewhat better than of timothy and smooth brome grass under these conditions. At the end of the three years, in plots seeded to simple mixtures, orchard grass occupied 52.5 percent of the stand where it had been seeded,

and Reed canary 45 percent, while timothy occupied but 15 percent and smooth brome grass but 5 percent.

Commercial hay timothy was used in these trials since, at the time the plots were seeded, no pasture strains were available.

The conclusion appears to be warranted that with respect to the strains used, neither timothy nor smooth brome grass will survive for any considerable length of time under close grazing or clipping, at least until strains better adapted for pasturing or for the climate are secured.

Project No. 41 - An Experiment in Topdressing Old Pasture Lands with Lime and Fertilizer. Seavey Pasture, Stratham, N.H.-

a. This test was continued through 1938 without change, although certain modifications in applications and time of topdressing were instituted in 1939. The figures for 1939 are not yet available, however, so we present here a seven-year average of the yields for the various plots up to and including the 1938 season.

The season of 1938 was marked by unusually heavy July rainfall and the yields for this year tend to make the averages higher for all treatments. Yields of dry matter on the check plots in 1938 approached one and one-half tons per acre, while certain treatments yielded well over two tons of dry matter per acre, one plot running almost two and one-half tons. This latter figure compares with slightly over one ton for the same treatment in the six-year average. It thus appears that summer rainfall lends considerable influence to the growth of grasses and clover in midsummer.

Seven-Year Summary, Seavey Pasture, 1932-38

Treatment	Plot No.	Average dry matter /acre	Percent protein	Average protein lbs./acre
555 lbs. basic slag, 3 yrs.	1	1924	16.8	323
No treatment	2-9-16	1740	14.9	260
Superphos.500 lbs. 20%, 3 yrs.	3	1724	17.1	295
" 167 " " , annually	4	1693	16.7	283
Mur.Potash, 200 lbs. 50%, 3 yrs.	5	1711	17.0	291
" " 67 " " , annually	6	1839	17.0	312
Nit. Soda 156 lbs. 16%, annually	7	2217	16.7	371
" " 312 " " , annually	8	2449	18.5	453
Superphos.500 lbs.; Mur.Potash 200 lbs., 3 yrs	10	2276	17.4	396
Superphos.500 lbs.;Mur.Potash 200 lbs. Nit.Soda 312 lbs.,annually	11	2521	18.3	462
Superphos.167 lbs.; Mur.Potash 67 lbs. Nit.Soda 156 lbs.,annually	12	2179	17.2	374
10-20-20, 500 lbs., 3 yrs. Nitrogen 50 lbs., annually	13	2458	17.6	432
Cyanamid 250 lbs.annually;basic slag 555 lbs.;Mur.Potash 200 lbs.3 yrs.	14	2595	16.7	433
Cal.Nitro 250 lbs.annually;basic slag 555 lbs.;Mur.Potash 200 lbs.3 yrs.	15	2577	17.9	462
Cyanamid 250 lbs., annually	17	2389	15.4	368
Sulphate of ammonia 250 lbs.,annually	18	2612	17.8	466

It can be noted in this table that all treatments increase protein percentages in the grass as well as total protein per acre, although the average check plot yields (2-9-16) are as high in dry matter as certain other plots, namely 3, 4, and 5.

One of the disturbing factors in a test of this kind and one over which the investigator has no control is the fact that fertilized portions of the pasture are always grazed more heavily than unfertilized portions. These plots are laid out side by side in the corner of a large field, with the check plots scattered amongst the treated plots. The cows come into this area and feed heavily as the grass is better there than anywhere else, manuring all the plots indiscriminately of course. Hence, we suspect that the check plot yields tend to come nearer the yields of other plots than they would if the plots were fenced and grazing controlled on each treatment. However, there seems to be no way to avoid this factor.

Comparing plots 3 and 4 it will be noted that the heavy triennial application of superphosphate on 3 gives slightly better results than the smaller annual application on plot 4. With potash on plots 5 and 6 this trend is reversed, the annual application being distinctly superior.

Plots 8, 17, and 18 receive the same annual application of nitrogen, 50 pounds, in the form of nitrate of soda, cyanamid and sulphate of ammonia respectively. The sulphate of ammonia and nitrate of soda plots yield somewhat more dry matter and considerably more protein and are higher in percentage protein than the cyanamid area.

The response for complete fertilizers when compared with equal amounts of nitrogen in the nitrogen alone plots is distinctly disappointing and differs materially from the results obtained on the Livingston Farm, data which will be subsequently discussed.

Amongst this data one can find very little consolation in the use of superphosphate alone or of complete fertilizers, although there a re indications in the trend of the data which would seem to point to the complete fertilizers outstripping the nitrogen alone plots, if the trend continues and if the experiment is extended for a longer period.

b. Livingston Farm Pasture, Claremont, New Hampshire. This experiment was modified somewhat in the spring of 1938 by applying complete fertilizers to plots that had previously received nitrogen only and vice versa. Hence, it is impossible to set down an average for all plots over the six-year period.

This soil is much more sensitive to a lack of minerals than Seavey pasture. Maximum yield are not secured with nitrogen alone, and the complete fertilizer plots yield more dry matter and protein than those treated with either phosphoric acid and potash or nitrogen.

Furthermore, on the plots which for five years had been treated only with nitrogen, clover appeared almost at once with the application of phosphoric acid and potash. The entire vegetation, pasture palatability, and length of season of pasturing were all favorably changed, so starved is the soil for phosphoric acid and potash.

For example, in 1938 the following data can be presented:

1938 Yields - Livingston Farm Pasture

Plot No.	Treatment	Dry Matter	Protein
28	50 lbs. nitrogen from nitrate of soda	3522	545
26	50 lbs. nitrogen from cal nitro	2882	439
2	50 lbs. nitrogen from nitrate with PK	5201	826
3	50 lbs. nitrogen from cal nitro with PK	5200	861

Plots 28 and 26 have received nitrogen only for the past four years, while plots 2 and 3 had received nitrogen only from nitrate of soda and cal nitro, respectively, for five years previous to 1938. In 1938, as has been noted, phosphoric acid and potash were applied to plots 2 and 3, and the yields increased materially as the data show. This increase was almost immediate, another important factor. The difference is certainly a significant one.

In view of the changes which were instituted on this pasture in 1938, and since the yields on all plots were much higher than they had ever been before, the data for all plots in 1938 will not be presented. However, the trends of the test can be followed in the next table which summarizes certain treatments that have been constant over a period of years.

Average yields - Livingston Pasture Plots

Treatment		Yield Dry matter per acre	Percent Protein	Total Protein per acre
No treatment	6-yr. ave.	1858	15.0	278
50 lbs. N. ave of two carriers	4-yr. ave.	2357	17.6	415
12-4-4, 408 lbs. per acre	6-yr. ave.	2607	15.9	416
0-20-20, 500 lbs. per acre	6-yr. ave.	2712	18.7	508
8-16-16 (H.M.) 625 lbs. per acre	4-yr. ave.	3838	19.1	734

The most interesting thing about the above data is the gradual stepping up of yields as more elements are supplied. Amounts of nitrogen are constant at 50 pounds elemental nitrogen per acre in all plots receiving this element. An application of nitrogen alone serves to increase yields and percentage protein in the forage as well. The amount of phosphoric acid in the 12-4-4 is too small to be of much benefit, indicating a higher need for these two elements than is carried by the 12-4-4 material. The 0-20-20 was applied annually at the rate of 500 lbs. per acre, except in 1938 when treatment was omitted. This material carried the same amount of phosphoric acid and potash as the 8-16-16-, except as noted.

These data serve to illustrate how responsive this soil is to phosphoric acid and potash. The main difference between the plots treated with these materials and those on which nitrogen only is applied lies in the amount of wild white clover which appears abundantly in the plots which are generously supplied with complete fertilizers or with phosphoric acid and potash.

New Jersey Agricultural Experiment Station

Department of Agronomy

Since pastures make up fully 30 percent of the total crop and pasture land on New Jersey farms, the research program on improvement of this important source of feed has been prosecuted vigorously. A 3-year comparison made between the system of soil improvement with the use of lime and a complete fertilizer, in comparison with lime, phosphate, and potash only, has shown that both types of treatment will double or treble feed yields. The cost of such feed by either system has been less than one-half the cost of purchased feeds for dairy animals. The lime, phosphate potash treatments which have greatly stimulated clover, produced feed at a considerably lower cost, since the nitrogen requirements of the pasture were provided by the clover. However, lime and a complete fertilizer high in nitrogen, produced pasturage at an earlier date, and the total yields for the year were somewhat higher. As a result of the research conducted at the College Farm, and on 25 cooperative pastures throughout the State, it has been concluded that both types of treatment have a definite place on the average dairy farm. Lime and complete fertilizer at the rate of 1,000 pounds per acre may be profitably used on a small proportion of the pasture for early feed and the treatment with lime, phosphate, and potash may be extended to the balance of the pasture. On farms where pasture acreage is small in proportion to the number of cows, the intensive treatment with lime and complete fertilizer may be applied to the entire acreage.

Experiments on the palatability of herbage with various treatments indicates that pastures in which clover is abundant, remain palatable throughout the year. Pastures in which clover is not abundant, and where growth has been stimulated by nitrogenous fertilizer, remain palatable until late june, but thereafter do not show as great palatability as plots containing clover. Of the various species found in pastures, all grasses are relatively unpalatable to livestock at the time seed heads are being formed, but become palatable to grazing animals after removal of seed heads by clipping. Since the various species produce seed heads at different times, 2 to 3 clippings during the course of the summer have been found necessary to maintain the pasture in a vegetative condition, palatable to livestock at all seasons.

White Clover Breeding.- Since clover plays an important part in determining the yield of pastures as well as their palatability and nutritive value, a breeding program on the improvement of white clover has been undertaken. The giant type of white clover represented by the commercial strain known as Ladino, has advantages over the native types of more dwarf nature, only when controlled grazing is practiced. These giant types are unable to endure continued close grazing throughout the summer. Study of plant types within a large number of commercial lots of white clover, and the examination of many native types, has indicated that giant forms may be found in nearly all of these, as well as forms of a dwarf nature. Great differences in the aggressiveness and yielding ability have been noted within the giant types as well as within the intermediate and dwarf types. Selection of superior plants of different types has proceeded, with a final choice of approximately 50 in the first group of families which have been under observation. Studies have likewise been made on the variability which occurs within families of white clover under restricted pollination in contrast with open pollination. This study is necessary to determine whether selection within

chosen families is adequate as a breeding procedure, or whether some more carefully controlled system must be adopted in order to isolate and fix desired characters in a given strain.

Department of Dairy Husbandry

Ladino Clover Studies.- From the trials to date at the Dairy Research Farm in Sussex, Ladino clover appears to be quite well adapted to the climatic conditions found there. During 1938 a yield of 28.6 tons of green weight per acre was reported. This plot was cut three times.

In the exceptionally dry season of 1939 acre yields compared very favorably with alfalfa yields on the same soil type. The Ladino clover withstood the drought conditions better than the native white clover in the same area. Entire areas of native white clover were burned out due to the dry weather. This was not true of the Ladino clover.

Another point of particular interest is that this clover seems to withstand winter conditions better than the native white clover and alfalfa. For two winters we have severe losses of native clover and alfalfa due to heaving. Practically no loss was suffered by the Ladino clover due to these same conditions.

Rotational grazing of Ladino clover seems to be the best type of management when this crop is grown for pasture. This system permits rapid recovery without injury to the root stocks. Recent grazing trials, the second summer after seeding, showed a yield of 354 one thousand cow days per acre or a carrying capacity for the grazing season of 2.48 one thousand pound animals per acre.

Ladino clover, because of its growth habit, seems to hold the weed population to a minimum.

From the trials to date, this plant seems to be a rich feeder. It responds well to applications of 250 pounds of super-phosphate and 200 pounds of muriate of potash per acre applied annually.

Belle Ellen Pasture Experiment - 1939.- The effect of different nitrogenous fertilizer treatments at varying rates of application on the botanical composition of the turf yield of clippings and yield of total digestible nutrients under rotational grazing management.

Ten pasture fields were used for this study. The area of these fields ranged from 3.2 to 7.2 acres. Out of the program of fertilization the pH values ranged between 5.3 to 6.3. All fields received 250 pounds of superphosphate per acre and 100 pounds of muriate of potash per acre annually. In addition to the basic application, two of the fields received an application of manure at the rate of 10 tons per acre. Seven of the fields received nitrogen fertilizer at rates of from 200 pounds to 500 pounds per acre.

Summary.- The prolonged drought very seriously curtailed yields during the season. The grasses were less palatable due to low moisture content and early maturity. The lack of palatability was indicated in

the relation of the grazing yields to the clipping yields in the area which showed that only 56 percent of the total clipping yields were utilized by grazing.

Nitrogen fertilization increased yields from 54 to 88 percent over the control plots. Native white clover largely disappeared from most of the pasture fields. The interesting fact was that these clovers were not only seriously depleted under the heavy nitrogen applications but also disappeared from the heavily manured fields and from the check fields as well.

The percent of clovers according to actual count by the Point Quadrat method averaged 22 percent in all the pasture areas when taken on May 9th. On October 9th the percentage of clovers had been reduced to an average of 2 percent.

Growth of Dairy Heifers Under Rotational Pasture Management.- During the season of 1938, 36 Holstein heifers gained an average of 192 pounds in weight and 6.8 cm. in height. Forty-two Guernsey heifers gained an average of 167 pounds with 4.0 cm. gained in height. These growth records were made on 180 days of pasture.

The effect of nitrogen fertilization on yields of timothy.- Five hundred pounds of sulfate of ammonia was applied on an old timothy sod on April 1, 1938. Seventeen thousand eighty-six pounds of green weight were harvested in the first cutting as against 6,812 pounds from the control area. This nitrogen application produced a 149 percent increase in green weight yield during the season.

Grass Silage Research.-

Preservation of grass silage - Citrus pulp was used as a carrier for molasses in the preservation of 200 tons of grass silage. The proportion of citrus pulp to molasses was 70 pounds of dehydrated molasses to 30 pounds of dehydrated citrus pulp. The product was non-hygroscopic. Approximately 100 tons of this product were added per ton of green material. Theproduct preserved the green material in good condition and due to the absorptive qualities of the citrus pulp, no seepage was observed to be lost in the silo. The silage was of good appearance and palatable to cows. This project will be continued another year and pressures within the silo will be studied.

Phosphoric acid preserved green material in a satisfactory manner. Green material such as mixed grasses and clover were ensiled, using 300 pounds of molasses per ton as the preservative. Some heating was observed in the top layers of the silage. However, after feeding off the top layer, the lower levels were of excellent quality. Analytical results have not yet been completed.

A study of silo pressures was made during the year and reported at the annual meeting of the American Society of Agricultural Engineers. Possibly two times the pressure of corn silage is observed in the preservation of grasses.

In studying the efficiency of grass silage as compared with corn silage for milk production, results indicate that the dry matter in mixed grass silage is at least equal in milk production value to the dry matter in corn silage and may be more efficient.

Temperatures developed in the two silos containing materials preserved with molasses and phosphoric acid, did not vary to any significant degree. Silos were filled simultaneously from the same field of grass.

Relation of the roughage diet of cows to the yellow color and flavor of milk -

a. The feeding of properly ensiled molasses grass silage produces a milk higher in total yellow color than does corn silage or beet pulp.
b. The yellow color of milk produced on molasses grass silage is more stable in storage than is that of milk produced on either beet pulp or corn silage.
c. First-calf heifers have a significantly lower milk color than do older, aged groups.
d. Milks high in yellow color show less actual loss and less percentage loss of color in storage than do milks of low-colored color.

Milk produced on a diet of molasses grass silage versus beet pulp and corn silage indicates that molasses grass silage is superior to beet pulp or corn silage in producing a milk which has a superior initial flavor, a more stable flavor in storage and a flavor more stable toward the catalytic effects of soluble copper.

Grass silage prevents oxidized flavor in milk.

Grass silage feeding seems to retard the development of hydrolytic rancidity.

New York (Cornell) Agricultural Experiment Station

Department of Agronomy

1. An investigations of methods for improving the quality and economy of production of feed crops in the principal soil and climatic regions of New York State (H. B. Hartwig, D. B. Johnstone-Wallace, and Richard Bradfield).

Outlying experimental fields have been established at Caldwell field on Dunkirk silty clay loam, and on the Mt. Pleasant Grazing Farm on the Lordstown and Volusia silt loam soils. Additional fields will be established at Marcellus on Honeoye silt loam and at other locations as soon as available.

In studies with Bird's-foot trefoil, it has been determined that while more acid tolerant than clover or alfalfa, this plant will respond to lime when growing on soil with a pH value of 5.0. Greenhouse studies indicate that seed should not be covered more than 1/4 inch. Data have been obtained relative to the range of soil conditions under which the plant has thrived naturally. Seedlings made in mid-summer have been fairly resistant to winter injury, but those made in October winter-killed. Under the latter conditions, however, there were enough hard seeds to provide some seedlings the following spring. Field results obtained to date indicate that Bird's-foot trefoil does

not establish itself as rapidly as clover or alfalfa. Field experiments covering rates of seeding, methods of seeding, time of seeding, seeding with different grasses and legumes, methods of management for both seed and hay production, and for pastures have been initiated.

In order to obtain improved pasture mixtures, especially for the poorer soils, seedlings of the more promising grasses and legumes have been made in Steuben County, Saratoga County, and at the Mt. Pleasant Farm. On the latter farm a 15-acre field has been seeded to grass and legume mixtures with two different fertilizer treatments. This field will be pastured by dairy heifers.

Twenty fertilizer tests consisting of broadcast applications of sixteen different combinations of lime, potash, phosphate and nitrogen at three different rates per acre have been set up. These tests are distributed over the main soil and climatic areas of the State on land furnished by cooperating farmers. The majority of the tests are on oats in which hay or pasture mixtures have been seeded. A few are made on corn and others on old-established meadows and pastures. These tests will be expanded in the future.

At the Mt. Pleasant farm a grass and legume nursery has been established in cooperation with the Soil Conservation Service. About 70 species and strains of grasses and 35 legumes have been planted in this nursery at three different levels of liming and fertility.

2. The development of a system of grassland farming adapted to the hill lands of southern New York (E. S. Savage, E. S. Harrison, Richard Bradfield, H. B. Hartwig, C. E. F. Guterman, and L. O. Bond of the Bureau of Agricultural Economics).

This project was outlined in the last Annual Report of the Regional Laboratory. The pasture research involved is a phase of that reported in the project above except that the work applies specifically to the class 1 and 2 lands of southern New York.

3. Pasture survey of New York (D. B. Johnstone-Wallace).

Practically all of the important pasture areas of the State have been surveyed. The data obtained in the several counties include botanical analyses of pastures on the important soil types, chemical analyses of herbage samples, response of pastures to methods of improvement and management, and variations in botanical composition when correlated with soil type, climate, topography, exposure, drainage, and fertilizer treatment. Much of the information accumulated during this survey has been published in Cornell Extension Bulletin 393, "Pasture Improvement and Management", and in Experiment Station Bulletins 536, 567, 570, 600, 612, 619, and 630.

During the past year, a preliminary survey was made of the areas in which New York wild Bird's-foot trefoil occurs in the State, especially in Columbia, Albany, Schenectady, and Saratoga Counties. Plants and seed were collected for further investigation. Attention was also given to the effect of recommended pasture improvement practices in various parts of the State.

4. A study of plant associations for pasture purposes (D. B. Johnstone-Wallace and J. A. Bizzell).

Grasses and legumes alone and in combination are grown in field plots and clipped with lawnmowers throughout the season to simulate close grazing. The total herbage so obtained is weighed and dried. Before drying, an adequate sample is taken and the clover separated from the grass by hand. Total dry matter, nitrogen, calcium, and phosphorus are then determined in the separate samples.

The species and varieties of grasses and legumes which have been especially outstanding under the conditions of this experiment were listed in the last Annual Report of the Regional Laboratory.

New York wild Bird's-foot trefoil appears very promising because of the high productivity recorded during a period of hot dry weather in July, 1938, when most grasses and legumes made very little growth. Although the data are not complete, this plant was again outstanding under the trying drought conditions of the present summer, 1939.

The experiments under this project have been expanded to include grasses and legumes adapted for the production of grass and legume silage and supplementary summer pasture. Experimental plots have been established on Caldwell Field and Willowbrook Farm, Cortland.

5. The management of uncultivated plant covers to conserve soil and water (D. B. Johnstone-Wallace, in cooperation with John Lamb, Jr., of the Soil Conservation Service).

The pasture phase of this project deals with grazing management, fertilization, and plant types in relation to soil erosion.

A series of plots on a 10 percent slope were seeded with Cornell pasture mixture. One plot was fertilized and lightly grazed, another fertilized and heavily grazed, and the third unfertilized and heavily grazed.

Another series of plots on a 23 percent slope, fertilized and unfertilized, were seeded, with and without wild white clover, to Kentucky bluegrass, Canada bluegrass, red top, Rhode Island bent grass, Seaside bent grass, pasture type timothy, timothy, orchard grass, poverty grass, or perennial ryegrass. The plots were subsequently grazed by sheep.

Under the conditions of these tests, the results indicate that even a fair grass cover will provide very effective control of soil erosion. The results are now being prepared for publication.

6. The production of silage from crops high in protein (J. K. Wilson).

This problem is being attacked by determining the neutralizing power of various forage crops high in protein for organic and mineral acids and by a study of the quantity of sugars such crops contain for the fermentative sugars which serve as a source of acids in the silo.

Silage from crops high in protein is now being made by the application of either molasses or liquid phosphoric acid. These materials aid the development of acids that prevent putrefaction. If the addition of phosphoric acid to the forage in silage-making continues to return good silage, the possibilities of this method are considerable.

Department of Animal Husbandry

1. Live weight gains per acre on purebred beef steers as given by improved New York State pastures (R. B. Hinman).

During each of the past five seasons, tests have been conducted to determine the amount of gain made by young beef steers per acre of improved pasture, developed from land that was too stony and rough for economical cultivation. The pasture was improved by cutting the brush and by fertilization with superphosphate.

The animals were turned on pasture in the spring as early as possible, averaging about the first of May, and were grazed without any grain or other additional feed until about August first. They were then fattened in dry lot for a period of 75 to 100 days on a liberal amount of grain with hay for roughage.

The gains per acre of pasture for the grazing period of about 90 days ranged from 135 pounds to slightly over 200 pounds, with an average of 167.5 pounds. This system of beef production seems especially suited to the farmer who wishes to make maximum use of winter roughages and of excess pasturage during the early part of the grazing season.

2. A comparison of various forage crops as temporary pastures for lambs (J. P. Willman).

During the summer of 1938, broad-leaved rape, thousand-headed kale and dwarf Essex rape were drilled in rows about 28 inches apart and were compared with broad-leaved rape sown broadcast. The crops in rows were weeded, hoed and cultivated once during the season. The carrying capacity of each crop was measured by recording the hours of sheep and lamb grazing that were furnished by each. When reduced to a percentage basis, the dwarf Essex rape produced 98.9 percent, the broad-leaved rape provided 70.7 percent, and the thousand-headed kale furnished 55.9 percent as much grazing when sown in rows as did the broad-leaved rape sown broadcast. These results do not agree with those obtained the previous year when the rowed-crop produced 88 percent more grazing than the same crop sown broadcast.

During the past six years, thousand-headed kale has ranked first four times and last twice in the amount of grazing furnished. Broad-leaved rape has ranked second during five seasons and first during one season. Dwarf Essex rape has excelled broad-leaved rape only once (1938) in six years.

3. The chemical changes taking place in phosphoric-acid silage, and the effect of this silage on acid-base relationships in the animal body (L. A. Maynard, E. Page, and W. L. Nelson).

Chemical studies have been conducted on the silage preserved with different amounts of phosphoric acid, including determinations of pH, total titrable acidity, total volatile acids, acetic and butyric acids, amino acids, and volatile bases. The layers of soybean silage

containing either no phosphoric acid or 8 pounds of phosphoric acid per ton developed a strong fermentation of the butyric-acid type. The values for pH, ammonia, volatile acids, and amino acids were very high in relation to those for the layers containing 16 or 24 pounds of phosphoric acid per ton. In a silage consisting of mixed grasses, the volatile constituents were rather low at all levels of phosphoric-acid addition but the amounts were inversely proportional to the amounts of acid added. About 50 percent of the volatile acids was butyric acid. The legume silage (90 percent alfalfa) had a very high moisture content when it was ensiled, and much settling and seepage took place. While the volatile acids were rather high in all layers, the percentage present as butyric acid was remarkably low. The amount of ammonia was inversely proportional to the amount of phosphoric acid added, but the total volatile acids decreased from the top to the bottom of the silo irrespective of phosphoric-acid addition. The data suggest that protein breakdown was fairly rapid during the first few days of ensiling. The data thus far obtained in these various studies indicate that no single determination affords a good measure of the quality of the silage.

Analyses were made of the blood and urine of four dry cows which were given corn silage and hay in one period, mixed-grass phosphoric-acid silage in a second period, and the phosphoric-acid silage plus legume hay or calcium carbonate in a third period. When the cows were changed from corn silage and hay to the phosphoric-acid silage alone, there was a marked drop in the pH of the urine, a nearly complete disappearance of fixed carbon dioxide in the urine, and a sixfold rise in urinary ammonia. When either hay or calcium carbonate was added to the ration, all of these values returned to the same levels as were found during the period when corn silage and hay were fed. While the feeding of phosphoric-acid silage alone produced these marked changes in the urine, there was no significant change in the carbon-dioxide-combining capacity of the blood plasma. The values for all the cows ranged between 56 and 67 volumes percent throughout the three periods.

4. The losses and changes accompanying ensiling of legumes and non-legumes with various amounts of phosphoric acid and the feeding value of phosphoric acid and molasses silage (E. S. Savage and E. S. Harrison).

During the summer of 1938 a number of silos were filled with legumes and grasses and various combinations of legumes and grasses, phosphoric acid being used as a preservative. The amount of phosphoric acid applied was varied with the kind of roughage being ensiled. The amount of acid applied was also varied with the same material. Rubber sheeting, sewed together and with seams waterproofed, was used to separate layers of silage in which different amounts of phosphoric acid had been used. The material was carefully weighed into the silo and the dry matter determined as the material was ensiled. These layers at feeding time were removed in entirety, weighed, sampled, and placed in another silo. This made it possible to measure rather accurately the losses that had accompanied the ensiling process. The silage as it was removed from the silos was examined also from the standpoint of quality, odor, spoilage, and palatability. A complete report on this experiment is not yet available.

A group of four cows, of which one was dry and three were in the latter stages of lactation, were fed phosphoric-acid silage as the sole diet. These cows lost weight rather rapidly and their condition became very unsatisfactory. They also tired of the phosphoric-acid silage, so that the consumption was greatly reduced. No definite conclusions can be drawn from this one trial. It is probably, however, that the condition of this group of cows was brought about by a disturbance of the calcium-phosphorus ratio.

During the comming year, molasses and phosphoric acid will be compared as a preservative for use in ensiling grasses and legumes.

Department of Entomology

1. White grub and other forage crop insect investigations (H. H. Schwardt).

In many areas of the State, white grubs (Phyllophage) are causing serious damage to pastures and meadows as well as to cultivated crops such as strawberries, raspberries, melons, corn, potatoes, nursery plants, and the like. A survey is under way to determine the extent of white grub injury, and the species involved. Experiments are also being conducted to determine satisfactory methods of control for infestations in the several crops affected.

With special reference to pastures, 175 plots have been established, in cooperation with the Department of Agronomy, in a heavily infested section of Steuben County. In these plots the repellent effect of several legumes and the influence of various fertilizer treatments are being studied. Data from these experiments are not available at the present time.

Department of Plant Breeding

1. Timothy breeding (C. H. Myers).

Approximately 70 different clonal types of timothy have been produced and are being maintained in field plots. Two of these have proved highly desirable for hay type in yield tests. Commercial seed sources of these two hay-type timothies are being established in New York, New Jersey, and Oregon.

Five pasture-type timothies have been selected from the clonal material. These types have been grown at the Soil Conservation Grass Nursery in Ithaca and small quantities of seed were obtained last summer. Some of this seed has been sent to other experiment stations for further testing and additional tests will be made at Ithaca.

Department of Plant Pathology

1. The disease factor in the problem of clover stands in the State of New York, with special reference to root and crown diseases (H. H. Whetzel, M. F. Barrus, and T. Sproston, Jr.).

A serious disease, caused by the fungus Sclerotinia trifoliorum and affecting chiefly the several clovers, has been discovered at Ithaca, New York. It is known that red, wild white, wild dutch, crimson, and sweet clovers are susceptible to the disease. It has also been determined that Bird's-foot trefoil is susceptible.

Although the disease has been reported from Kentucky, Tennessee, Montana, and Canada, its presence elsewhere in New York State has not been verified.

Attempts to obtain plants resistant to the disease have met with some success - especially with red clover. A selection of this plant which appears to be immune is being subjected to further tests.

A technique for testing susceptibility of seedlings to this fungus on agar in flasks has been developed. This method promises to materially speed up and accurately sort out resistant or immune individual plants in seed lots of clover or other suscepts.

Department of Poultry Husbandry

1. Poultry pastures (G. F. Heuser, in cooperation with D. B. Johnstone-Wallace).

This project has as its objectives the determination of the value of pastures for poultry from the standpoint of types of grasses, sanitation, and nutritive value.

Dry weather during the past three summers has hampered the work to the extent that satisfactory seedlings for range purposes have not been attained. The grasses used were: Kentucky bluegrass, Canada bluegrass, rough-stalked meadow grass, timothy, Welsh pasture timothy No. S-50, New Zealand certified perennial ryegrass, red top, Rhode Island bent, and Festuca arenaria. All of the above were seeded alone and in association with wild white clover.

Information has been accumulated relative to the composition of various grasses. These records include analyses for protein, ash, dry matter, calcium, phosphorus, and manganese. Part or all of this information is available for approximately 40 samples.

A preliminary experiment is under way to determine the influence of grass upon hens being fed a ration deficient in the vitamin G complex.

Pennsylvania Agricultural Experiment Station

Project No. 777 - Pasture Fertilization-

Pasture fertilization studies which have been conducted in Clear-

field County for the past eleven years in cooperation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, were terminated in the fall of 1939. The results of the first five years, including the season 1934, were published in Bulletin 323. The 1938 and 1939 pasture seasons have been very unsatisfactory because of the drought. The results of these two seasons will make no material change on the results reported for previous seasons.

Returns per acre from Kylertown, Pa., Pasture Project No. 777 - 1934 to 1937

Treatment*		Pounds 4% milk	Value of milk $2.00 per 100#	Cost of concentrates fed $1.50 per 100#	Cost of fertilizer	Returns above cost of conc. and fert.
L	1934	755	$ 15.10	$ 3.13	$ 0.00	$ 11.97
	1935	959	19.18	3.49		15.69
	1936	986	19.72	3.18		16.54
	1937	1107	22.14	4.27		17.87
LP	1934	1576	31.52	5.66	1.92	23.94
	1935	3053	61.06	10.62		48.52
	1936	2187	43.74	7.12		34.70
	1937	2136	42.72	9.97		30.83
LPK	1934	1471	29.42	5.67	3.17	20.58
	1935	2275	45.50	8.58		33.75
	1936	2611	52.22	8.10		40.95
	1937	2864	57.28	11.42		42.69
LPKN	1934	1643	32.86	5.87	5.51	21.48
	1935	1984	39.68	7.09		27.08
	1936	2313	46.26	7.14		33.61
	1937	2103	42.06	9.04		27.51
LPKN2	1934	1091	21.82	4.49	5.75	11.58
	1935	2860	57.20	10.23		41.22
	1936	2306	46.12	7.32		33.05
	1937	2411	48.22	9.43		33.04
LPK2N	1934	2161	43.22	8.83	7.97	26.42
	1935	2129	42.58	8.15		26.46
	1936	2705	54.10	8.43		37.70
	1937	3023	60.46	12.01		40.48
LPK2N2	1934	1735	34.70	7.12	8.15	19.43
	1935	2156	43.12	8.52		26.45
	1936	2804	56.08	8.69		39.24
	1937	3249	64.98	14.00		42.83
LPK3N2	1934	2236	44.72	9.34	10.45	24.93
	1935	3072	61.44	11.91		39.08
	1936	3104	62.08	10.47		41.16
	1937	3647	72.94	15.60		46.89

* N = 24 lbs. nitrogen in sulphate of ammonia.
P = 64 lbs. phosphoric acid in superphosphate.
K = 50 lbs. potash in muriate.

A new pasture management and fertilization project is planned to take the place of the old Kylertown project. The cooperation with the U. S. Department of Agriculture (Division of Forage Crops and Diseases) is to be continued. A representative dairy farm with rather complete equipment, located in the Volusia soil area near Montrose in Susquehanna County (about 14 miles from the New York State line), has been leased for a ten-year period.

Approximately 50 acres will be devoted to permanent crop rotation pastures. Forty to fifty acres of additional land will be available for cultivated crops. It is the plan for the College and the U. S. Department of Agriculture to equip the farm completely with livestock and implements. A total of 50-60 head of high grade Holstein cattle will be maintained on the farm continuously, this number to be made up of 25-30 head of milking cows and the usual young and dry stock. Some of the management practices will include grass and legume silage. The farm is to be equipped with large and small silos.

The first year will be devoted largely to preliminary work, but it is hoped that some phases of experimentation can be put under way in the spring of 1940. A definite experimental outline will not be decided upon till the fall of 1940.

Two new projects have been planned and preliminary work on each of them has been put under way during the past year.

1. Title: Investigation of Snowmold on Pasture and Fine Turf grasses. Project No. 959.

Objective:
a. To standardize inoculation under controlled conditions.
b. To determine the host range of the various species with respect to economic turf and pasture grasses.
c. To select disease resistance strains of susceptible species of turf and pasture grasses.
d. To determine the occurrence of physiologic races of the causal organisms and methods of origin.
e. To study the physiology of the organisms and hosts under natural and artificial conditions.

It is expected that the project will be expanded to include the effect of environmental conditions on the interaction between the host and parasite, together with the more indirect effect of such conditions as temperature, humidity, fertility, management practices, etc.

Leaders: C. C. Wernham, Pennsylvania Experiment Station, and S. J. P. Chilton and V. G. Sprague, U. S. Regional Pasture Research Laboratory.

2. Title: Breeding Kentucky Bluegrass (*Poa pratensis* L.) for Pasture Types. Project No. 960.

Objective:
a. To study methods and technique for breeding Kentucky bluegrass.

b. To breed strains of the species.
c. To measure the value for pasture purposes of new and existing strains by suitable production tests.

Leaders: H. B. Musser and S. I. Bechdel, Pennsylvania Experiment station, and W. M. Myers, U. S. Regional Pasture Research Laboratory.

Work has progressed satisfactorily on the following projects, but the leaders in charge have nothing to add to the material reported in the first and second annual reports:

Penna. Station - Project 773 - Testing and Breeding Strains of Clover. H. B. Musser, Leader.

Penna. Station - Project 805 - Testing and Breeding Grasses for Pasture and Other Uses. H. B. Musser, Leader.

Penna. Station - Project 889 - The Effect of Different Systems of Grazing, Cutting, and Fertilization of White Clover in Permanent Pastures. H. B. Musser, Leader.

Penna. Station - Project 806 - Growth and Maintenance as Influenced by Various Soil Factors, Fertilization, and Methods of Management. J. W. White, Leader.

Penna. Station - Project 961 - The Preparation, Fermentation, and Feeding Value of Grass and Legume Silage. S. I. Bechdel, R. W. Stone, et al. (In cooperation with the Division of Bacteriology and the Division of Agricultural and Biological Chemistry.)

Crops worked on to Date - First, second, and third cutting alfalfa, soybean forage, and wheat.

A comparative study was made last year on molasses and phosphoric acid as preservatives for two crops, alfalfa and soybeans. These materials preserved the silage satisfactorily. In most instances the molasses silage was somewhat superior in palatability, but the carotene and other nutrients were preserved equally well. In milk production tests, the silages (molasses vs. acid) proved to be about equal in feeding value, but in long continued feeding the acid silage brought on several cases of severe malnutrition. The phosphoric acid was suspected of drawing calcium from the body tissues. We are definitely of the opinion that pulverized limestone or some other form of calcium should be fed with acid legume silage.

Extensive studies are progressing at this writing on second cutting alfalfa preserved as follows:

Silo No. 1 - Preservative - 120 lbs. molasses per ton
Silo No. 2 - Preservative - 80 lbs. molasses per ton
Silo No. 3 - Preservative - 18 lbs. P_2O_5- 73 percent per ton

Silo No. 4 - Preservative - 40 lbs. molasses per ton
Silo No. 5 - Preservative - 40 lbs. molasses and 9 lbs. acid per ton
Silo No. 6 - Preservative - 40 lbs. molasses and 18 lbs. acid per ton

Fermentation studies (bacteriological and chemical) have been in progress since storage in September. Feeding tests are also under way with cows.

Various blood and urine studies are being made to determine the possible harmful effects of long continued feeding of acid silage. This study has also been planned to answer more fully the question of how much preservative to use for best results.

Penna. Station - Project 918 - Alfalfa Molasses Silage as a Roughage for Fattening Steers. P. T. Ziegler, F. L. Bentley, et al.

In the seasons of 1938-39 three lots of steers (a total of 60) were fed in such a way as to compare corn silage, Lot I; alfalfa molasses silage, Lot II; and alfalfa hay, Lot III; as roughages.

The following results are of interest:

	Lot I		Lot II		Lot III	
	1938	1939	1938	1939	1938	1939
Average daily gain, pounds	2.08	2.08	2.36	1.94	2.13	1.94
Return per cwt. of corn and cob meal fed	$.82	$.91	$.92	$.85	$.76	$.88

Further feeding trials are in progress at this writing. The results to date indicate that alfalfa molasses silage is a very satisfactory roughage for fattening steers, but it appears to offer little if any advantage in economy of beef production.

Rhode Island Agricultural Experiment Station

Purnell Project - A Study of the Adaptability of Sudan Grass, Japanese Millet, Oats, Winter Rye, and Winter Wheat for Supplementary Pasture. T. E. Odland and T. R. Cox, Leaders

Sudan grass, Japanese millet, oats, winter rye, and winter wheat were considered for the second year as pasture crops to supplement the normal pasture shortage during the spring and late summer months. Comparisons were made of different seeding dates for all crops. Sudan grass was planted in both loose and firm seed beds, and comparisons were made of four different heights of cutting from one to four inches above ground level.

Rye planted August 30, September 10, and September 20, yielded pasturage from about April 15 to May 25, producing about six tons of green rye pasturage per acre during this period. Winter wheat planted at the same dates yielded from on-half to two-thirds as much.

Oats planted April 15 yielded pasturage from June 1 to July 1. When planted on April 25, it was cut four times between June 1 and July 15. When planted on May 5, it was cut three times from June 6 to July 3. The total yield was about 4.5 tons green weight per acre from the April 15 planting, 7 tons for the April 25 planting, and 3.5 tons for the May 5 planting.

Sudan grass and millet planted at two-week intervals from May 20 to July 15 furnished grazing from July 1 to September 1. The total yield varied from 1.5 to 7.5 tons of green material per acre depending on the time of planting and the preparation of the seed bed. Plantings made June 1 and June 15 produced the best yields at the season when supplementary pasture was needed the most. The well-firmed seed beds with the July 3 and July 15 plantings yielded more than twice the amount obtained from the loose seed bed planting. There were only small differences in favor of the well-firmed seed bed when moisture was more ample early in the season.

Adams Project - Studies of Growth Habits of Pasture Grasses as Related to Environmental Factors and Fertilization. B. E. Gilbert and I. H. Stuckey, Leaders.

Response of Pasture Grasses to Environmental Factors.- This investigation is a continuation of the experiments carried on during the previous winter with special emphasis on photoperiodism as related to temperature. The plants were grown in beds in a greenhouse kept at 67°F. from February to June. The day lengths, respectively, were 16 hours (long day), 8 hours (short day), check (normal varying day length.) Half of the seedlings were grown continuously in the greenhouse and the others were exposed to freezing temperatures out of doors for ten days before being transplanted into the beds. Observations were made of the vegetative and reproductive growth.

Most of the grasses could be classified definitely as long-day, short-day, or indifferent, but the reaction to a given photoperiod was considerably influenced by temperature. The response of Kentucky bluegrass was completely masked by a temperature of 60°F. Rough-stalked meadow grass is apparently a short-day plant, but the production of inflorescences was too irregular to be definite. The crested wheatgrass also was not so vigorous as the others and can be classified only tentatively as a long day plant. Some of the irregular blooming among these species of grasses may be due to genetic differences in the original seed, since pure line strains were not used.

Both perennial ryegrass and Italian ryegrass bloomed under all light conditions, but flowering occurred sooner with the 16-hour day. Commercial timothy also bloomed with all light conditions, but the flower heads produced after the cold treatment w ere abnormally long. Canada bluegrass and timothy S-50 responded to a long day. Blooming was materially hastened by the cold treatment. Reed Canary grass and orchard grass both bloomed with a short day. They grew vigorously with the 16-hour day, but never produced flower primordia. Short-day plants exposed to low temperatures bloomed la ter than those grown altogether at 60°F.

The last four grasses named were planted under the conditions described above in September, 1939, for further experiments.

A study of the cell size of vegetative plants of orchard grass suggests a correlation between the length of cell elements and length of day.

Response of Pasture Grasses to Moisture.- The moisture relations of pasture grasses is another phase of the effect of environmental conditions which has been continued from last year. Clones of Kentucky bluegrass, timothy S-50, crested wheatgrass, and red top were transplanted into the pans July 15. One-half of the pans were maintained at 25 percent moisture and the other half with 15 percent moisture. Two pans of each species with each treatment were clipped beginning August 28, a third was left as a control. Growth as measured by clipping, was rapid at first and then gradually decreased until at harvest (November 17) there was no measurable growth.

In general, the total yield for each species was the same whether clipped or unclipped within a given moisture treatment. There were differences, however, between the weights of tops produced by plants growing with 25 percent moisture and with 15 percent moisture. Kentucky bluegrass was the most sensitive. Next in order, came timothy S-50, red top, and, finally, crested wheatgrass.

The parts below ground responded more than the tops. The rhizomes on the Kentucky bluegrass were largest and most numerous in the pans grown with 25 percent moisture, the unclipped pan being much superior to the other two. The development of the roots of all species was of the same magnitude as that of the rhizomes of Kentucky bluegrass. The roots were more dense and more new roots were present where the plants had not been clipped. The root systems were much more vigorous with 25 percent moisture than with 15 percent moisture.

Timothy S-50 was the only grass to bloom during this experiment. The plants with low moisture sent up spikes a week before the others and the pollen was produced a week earlier, also. On these plants the seed heads developed normally and produced viable seed. Those plants growing with 25 percent moisture, however, produced 50 percent semivegetative inflorescences. The lemmas continued to grow and resembled small leaves several millimeters long. These leafy heads remained green and vigorous long after the normal heads had died. Among these leafy spikelets, normal spikelets containing viable seeds were produced. The production of these abnormal seed heads was possibly related to the photoperiod. Reproductive growth was initiated during the long days of mid-summer, but the final development was sufficiently influenced by the shorter days of September causing the reversion to vegetative growth. Water was the limiting factor on those plants grown with 15 percent moisture inhibiting the influence of the shorter days.

Root Development of Pasture Grasses.- Some observations of the growth of roots on grass plants at low temperatures were made during the winter and spring, using both plants grown out-doors and in flats in the pot house. The temperature of the latter averaged 10° higher than out-doors.

The grasses used were:

Red top	Agrostis alba
Kentucky bluegrass	Poa pratensis
Canada bluegrass	Poa compressa
Reed Canary grass	Phlaris arundinacea

Orchard grass	Dactylis glomerata
Rough-stalked meadow grass	Poa trivialis
Crested wheatgrass	Agropyron cristatum
Commercial timothy	Phleum pratense
Timothy S-50	Phleum pratense var. S-50
Italian ryegrass	Lolium multiflorum
Perennial ryegrass	Lolium perenne
Meadow fescue	Festuca elatior

New roots developed continually in the pot house throughout the winter, even when the soil was frozen. Out-of-doors, root development ceased altogether during January, but was resumed in February. Sections of root tips showed a few mitoses in timothy S-50 and meadow fescue. They occurred more frequently in red top, rough-stalked meadow grass, commercial timothy, and Kentucky bluegrass. Two weeks later the soil had thawed and root growth was very active in all the species. In some species the roots of the previous season continued to grow and in these plants the new roots produced from the crown were not so numerous as in thos species with annual roots. The maximum production of new roots from the crown occurred during the last week in March. Maximum growth took place during the first week of April. There was very little development, either production of new roots or extension of existing roots after May 1, at which time flower primordia were being formed. A month later, after heading had occurred; further new growth was observed, but whether it was associated with the heading or due to the added water after a long drought was not determined.

Vermont Agricultural Experiment Station

Fall Versus Spring Application of Nitrogen on Pasture and Meadowland.- Nitrogen applied in the spring has given slightly greater yields of pasture and hay than similar applications in late fall. However, in case of urea and cyanamid application on a good turf, the differences were not very great. Cal-nitro (nitrate) showed greater differences in this respect and results indicate that care should be exercised in the use of this type of nitrogen in late fall.

Cyanamid may give some "burning" even with late fall applications. This is especially true on heavy clay soils that are too cold for proper chemical and biological changes of the cyanamid to take place.

Effect of Rare Elements on Growth of Native Clover in Pastures.- A series of test plots have been set out on two soil types (sand and clay loam) to study the effect of magnesium, manganese, and borax on the establishment and maintenance of natural white clover pasture. Data are available for only one year but results indicate that boron may be quite beneficial, in addition to the minerals, on the lime plots. Without lime, boron gave little if any response. The same has been true for alfalfa and a number of other crop plants. Heavy rates of lime greatly reduce the availability of boron.

Pasture Seeding Mixtures.- Studies on this project have been enlarged to enclude zigzag clover, New Zealand clover, bird's-foot trefoil, and some western grasses, especially bromegrass. Zigzag clover resembles ordinary red, but it has running root stocks and it is perennial. It has been estimated that a field of zigzag clover near Chelsea, Vermont, has been there for fifty years and at the present time it extends over five acres. Root cuttings from this field, together with a number of selected strains from the Bureau of Plant Industry, Washington, D. C., are being propagated for more detailed studies later on.

Conservation of Nutrients in Roughages.- Experiments relative to conserving nutrients in roughages are still in progress at this Station. Crops of timothy, alfalfa, red clover, and soybeans have each been preserved as silage, sun-cured hay, and artificially-dried hay. Losses of nutrients occurring in these different processes have been compared on the timothy and alfalfa. The results indicate that artificial drying conserves the most total digestible nutrients and that there is no great difference in the preservation by the silage and sun-curing methods, provided the weather is ideal for haying. Carotene seems to be retained best in the artificially-dried hay, poorest in the sun-cured and intermediate in the silage.

Various methods of preserving the crops as silages have been tried - the addition of molasses, phosphoric acid, mineral acids as in the A. I. V. procedure, and no preservatives. It was found possible to obtain a satisfactory silage without the addition of any preservative provided the dry matter content was satisfactory (around 30-40 percent) but in order to insure against variations occurring under farm conditions it is deemed safer to add a preservative. Molasses is preferred to mineral acids as the acid silages are not especially relished by the animals and in most cases lime must be fed to offset the excess acidity.

Digestion trials have been conducted for four years on all lots of silages and hays. The various crops properly preserved either as hays or silages showed no outstanding differences in digestibility.

Feeding trials with heifers were made to compare the value of timothy preserved by three different methods, namely, ensiling, sun-curing, and artificially-drying, with that of corn silage. Based on digestible nutrients consumed per pound of gain, the groups ranked in the following order: (1) artificially-dried timothy hay, (2) sun-cured timothy hay, (3) corn silage, and (4) grass silage.

Also, phosphoric acid preserved silage is now being fed to growing heifers in comparison to sun-cured hay.

West Virginia Agricultural Experiment Station

1. Studies on West Virginia Pastures: I. Pasture Fertilization Studies. F. W. Schaller, G. G. Pohlman, H. O. Henderson, R. A. Ackerman, and E.A. Livesay, Leaders.

Small plot experiments.- Yield data and botanical estimates showing residual effect of fertilizers and lime on different soils in four

locations continue to show beneficial effects from the use of phosphorus and lime. The increases in yield on the plot receiving lime and superphosphate over the limed plot varied from 13 to 43 percent in the various areas. Increases for lime varied from 0 to 12 percent. Some of the plots showed a slight increase as a result of the application of potash fertilizer. Nitrogen showed an effect on all plots, the most marked effect being noted on those plots which had only a small percentage of legumes. In addition to the effect on yield, the fertilizer and lime treatments brought about an increase in the content of desirable pasture plants.

In the spring of 1937 some of the check plots were treated with lime and superphosphate and a few had seed in addition. The addition of lime and 500 pounds of 20 percent superphosphate increased the desirable species from 2 percent in the untreated plots to 15 percent in the treated plots. Seed, in addition to lime and superphosphate, increased the percentage of desirable species to 23 percent, most of the increase being the result of additional clover. The yield of forage was increased by about 25 percent, there being very little difference between the plots which had seed and those which did not.

- Grazing experiment.- These plots continue to show a correlation between yields obtained by clipping and by grazing. The yield of total digestible nutrients based on 100 for the grazing method were 67 for the permanent plots, 76 for plots relocated each year, and 83 by the difference method.

Time of nitrogen application to pastures.- Nitrate of soda applied at the rate of 200 lbs. per acre gave an increase of 47 percent in the yield of clipped herbage. There was very little difference in total yield as a result of different times of application. However, on those plots which received part of the nitrogen in June or July the production was higher during the latter part of the season. The data for this experiment have been summarized and will soon be submitted for publication.

Residual effect of lime and fertilizers.- Lime applications in 1923 and superphosphate in 1927 are still showing a marked residual effect on the botanical composition of an old established pasture. There are only 10 percent desirable species on the untreated plots as compared to 34 percent on the limed plots and 41 percent on those receiving lime and superphosphate. Very little difference was evident in the yield of clipped herbage of the various plots. Soil samples taken in 1937 show that the lime has penetrated about 7 inches, but the effect is most marked near the surface.

Movement of lime and fertilizer.- Soil samples have been collected from a number of the plots at depths of 0 - 1-1/2, 1-1/2 - 3, 3 - 5, and 5 - 7 inches to study movement of lime and phosphate. A few of the results showing available phosphorus content at the various depths are shown in the following table:

Soil type and treatment	Available phosphate[3] p.p.m./2,000,000 pounds		
	0 - 1-1/2	1-1/2 - 3	3 - 5
Dekalb silt loam[1]			
Check	27	9	8
N-P-K-L	53	12	9
N-2P-K-L	128	29	9
Monongahela fine sandy loam[2]			
Check	23	16	16
N-P-K-L	91	28	23
N-2P-K-L	106	40	14
Upshur clay loam[1]			
Check	16	7	7
N-P-K-L	21	9	9
N-2P-K-L	66	18	8

[1] Fertilizer applied at rate of 1000 pounds of superphosphate per acre for P treatment, one-half in 1930 and one-half in 1932. The 2P treatment consisted of 2000 pounds of superphosphate applied in four equal applications in 1930, 1931, 1932, and 1933.

[2] Fertilizer applied at rate of 1000 pounds of superphosphate per acre for P treatment, one-half in 1931 and one-half in 1933. The 2P treatment consisted of 2000 pounds of superhposphate applied in four equal applications in 1931, 1932, 1933, and 1934.

[3] The soil samples were taken in the spring of 1937, fall of 1937, and spring of 1938 for the Upshur, Dekalb, and Monongahela, respectively.

The studies on downward movement of lime show that there has been some effect in the lower layers in the limed plots that the effect is most marked in the surface. The results of the Dekalb silt loam soil are summarized in the following table:

	Unlimed	Limed	Change
0 - 1-1/2"	4.95	6.18	+1.23
1-1/2 - 3"	4.97	5.65	+ .68
3 - 5"	5.13	5.41	+ .28
5 - 7"	5.39	5.49	+ .10

These samples were taken seven years after the initial application of lime. There is a little evidence to indicate that lime movement has been greater on the plots receiving nitrogen fertilizer than on those which did not.

2. Studies on West Virginia Pastures: II. Pasture Management Studies. G. G. Pohlman, F. W. Schaller, and E. A. Hollowell, Leaders.

The results of the first two years show a marked decline in white clover when cut twice during the season at the hay stage. Clipping three inches high in the spring, followed by 1-1/2 inch clipping during the latter part of the season has also reduced the clover content

as compared to 1-1/2 inch clipping over the entire season. No difference was noted between the three strains tested. This fall it was noted that crab grass was much less prevalent in the plots which were clipped 3 inches high in the spring than in those clipped 1-1/2 inch throughout the season.

3. Supplementary Pastures for West Virginia Livestock. H. O. Henderson, R. A. Ackerman, and G. G. Pohlman, Leaders.

4. The Effect of Contour Pasture Furrows on the Conservation of Soil and Water. G. M. Browning, F. W. Schaller, and G. G. Pohlman, Leaders.

Objectives: To determine the effect of contour pasture furrows on-
a. The type and amount of vegetation produced over a period of years.
b. The amount and distribution of soil moisture.
c. The efficiency and economy of contour furrows in comparison with other approved pasture management practices as a measure of improving pastures and conserving soil and water.

These plots were started in the fall of 1939 and no results are available.

Publications:

"Response of Permanent Pastures to Lime and Fertilizers (1930-1936)". W. Va. Agr. Expt. Sta. Bul. 289. R. R. Robinson and W. H. Pierre.

Subject Index to State Progress Reports

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Appendix B

REPORT OF COLLABORATOR'S MEETING

The collaborators on pasture research of the Northeastern States held their third annual meeting at the U. S. Regional Pasture Research Laboratory, State College, Pennsylvania, on October 3 and 4, 1939. Representatives were present from all States except New Hampshire. In addition to the collaborators the following non-resident members of the United States Department of Agriculture attended the meeting: O. S. Aamodt, E. A. Hollowell, and M. A. Hein, of the Division of Forage Crops and Diseases, and H. W. Marston, of the Office of Experiment Stations. All or some of the sessions were attended by T. E. Odland and Irene H. Stuckey of the Rhode Island Agricultural Experiment Station and Director S. W. Fletcher and C. F. Noll of the Pennsylvania Agricultural Experiment Station.

During the forenoon and early in the afternoon of the first day the discussions were concerned primarily with progress in pasture research conducted during the past year at the State experiment stations. Each collaborator distributed to those present a short mimeographed report covering the more significant accomplishments.

Following these presentations the question of increasing the usefulness of the annual State reports included in the annual report of the Laboratory was raised. After some discussion the following form was adopted for the 1939 reports.

Collaborator responsible for-

1. Name of station.
2. Project titles and leaders
3. Brief report of last year's progress under each project. (If new project, statement of objectives and plan of procedure.)

Laboratory responsible for-

1. Index for combined reports to allow ready reference to particular subjects under each station's report.

It seemed to be the concensus of opinion among collaborators that a brief report of grass silage research under way in the Northeastern States should be included with the pasture reports.

During the remainder of the afternoon until 4:00 o'clock, when the meeting adjourned to permit anyone wishing to visit the plant nursery to do so, general problems were discussed, particularly from the standpoint of ways and means of bringing about a close coordinated effort in their solution. "Methods of measuring results from pasture experiments" and "Effects of placement of lime and fertilizer on soil and plants" were problems emphasized in this connection. It was suggested that the possibilities of setting up cooperative projects along those lines be explored. The general interest in the first problem mentioned was apparent.

During the past year three cooperative breeding projects and one pathological project have been set up between certain State stations and the Laboratory and a few more are contemplated. A copy of the project outline covering the breeding of Kentucky bluegrass to be carried on cooperatively by the Pennsylvania State College and the Laboratory was given to each person attending the conference.

The special order of business for the evening session - 7:00 p.m. to 9:00 p.m. - was the presentation by Dr. Noll of tentative plans for a rather extensive pasture management experiment to be carried on near Montrose, Pa., by the Pennsylvania Agricultural Experiment Station and the Division of Forage Crops and Diseases. The presentation of the plans was followed by a general discussion during which numerous suggestions were made. After plans for this experiment are further developed interested persons of the Pennsylvania and New York Agricultural Experiment Stations and of the Division of Forage Crops and Diseases will hold a conference, possibly at Montrose. Drs. Fletcher and Guterman will make the preliminary arrangements for the meeting.

The discussion of new projects or the modification of old projects was continued the next morning. In this category projects from

Maryland, Rhode Island, Connecticut, West Virginia, and Delaware were mentioned and considered briefly. It was agreed that outlines of these proposed projects or modification of old projects would be sent to each collaborator for suggestions and criticisms. It was further agreed that the collaborator located at the station where the project originated would send the outline to each of the other collaborators and they in turn would return any suggestions of their own or associates in pasture research.

Following these discussions, consideration was given to the nature of the program that should be arranged for next year.

It was proposed that progress reports might be greatly curtailed or eliminated and that a program be arranged around a few general subjects, particularly subjects of general regional interest and therefore most likely to engender cooperative research. Below, the topics - two of which were mentioned earlier - are listed:

1. Effects of placement of lime and fertilizers on soil and plants.
2. Methods of measuring results from pasture experiments.
3. Strain testing.
4. Pasture management.
5. Weeds in permanent pastures.
6. Grass silage.
7. Ways and means of accomplishing more breeding work.

It was suggested that next year's program for the collaborators' meeting be built around one or more topics of particular interest to those concerned with environmental pasture problems; one or more to those concerned with breeding problems; and one or more topics of general interest to the group as a whole. Insofar as practicable, each collaborator will undertake to bring with him to next year's conference his experiment station associates who may be particularly interested in the topics to be discussed.

The final session of the meeting consisted of a brief presentation of progress reports by members of the Laboratory staff.

Adjournment at 4:00 p.m.

Appendix C

REPORT OF NORTHEASTERN PLANT BREEDERS' MEETING

The second conference of persons interested in breeding pasture plants in the Northeastern United States was held June 27 at the U. S. Regional Pasture Research Laboratory, State College, Pa. The following State representatives were in attendance: R. A. Emerson, New York; W. B. Kemp, Maryland, H. B. Sprague, New Jersey; H. B. Musser, Pennsylvania; W. G. Colby, Massachusetts; E. J. Wellhausen, West Virginia; and C. E. Phillips, Delaware. S. S. Atwood, W. M. Myers, W. H. Brittingham, and R. J. Garber, of the Laboratory Staff, were also present. The conference began shortly after 8:00 o'clock a.m. and continued throughout the day and evening until adjournment about 10:00 o'clock p.m.

The forenoon was spent examining and discussing the plant material in the nursery and greenhouses. By following this plan it was possible to acquaint all the delegates with the current activities related to breeding that the Laboratory is carying on. The visiting group expressed particular interest in the sterility studies under way with white clover and some of the grasses; the sod plots where an attempt is being made to evaluate certain grasses associated with clover and vice versa; and the differential reaction of clones in greenhouse cultures to fertilizer treatment, clipping treatment and water utilization.

In the afternoon the conferees divided themselves into two groups depending on their primary interest - clover breeding or grass breeding - in order to discuss ways and means of bringing about a more aggressive breeding campaign and more effective cooperation among State and Federal agencies in this campaign. After about two hours of discussion the two groups reassembled.

All agreed that the Laboratory should continue to engage in research designed to reveal facts related to breeding rather than to engage in breeding. The primary responsibility for the actual breeding of improved strains should be assumed by the State stations. However, this work should certainly be coordinated and in some cases it may be desirable to set up cooperative projects either among State stations or between State stations and the Laboratory.

The group expressed themselves as favoring the idea of each Station limiting its major breeding activity insofar as pasture plants are concerned to one or two species. It was agreed that more would be learned about the possibilities of improving pastures by breeding in the Northeastern Region if each station interested would conduct an aggressive breeding program with one or two species rather than to breed less intensively a larger number of species. Preferences were expressed for the following species by the State stations indicated:

Delaware - Lespedeza
Maryland - Orchard grass and ryegrass
Massachusetts - Ladino clover
New Jersey - White clover
New York - Timothy
Pennsylvania - Kentucky bluegrass
West Virginia - Kentucky bluegrass

Trials under local conditions will be necessary to determine the value of any new strains.

A number of delegates stated that they were handicapped in expanding their grass breeding program because of lack of funds. Additional technical assistance, such as provided by graduate students or technicians, would be particularly helpful in a breeding program involving artificial pollination. Some also reported inadequate labor to care for an extensive individual plant nursery.

In the evening session a proposed cooperative orchard grass breeding project between the Maryland Agricultural Experiment Statien and the Pasture Laboratory was discussed. Dr. Kemp agreed to draw up a project and after details were worked out with the Laboratory to submit the project for approval in the usual manner.

Pending the availability of certain State funds, Dr. Sprague agreed to draw up a similar white clover project involving the New Jersey Agricultural Experiment Station and the Laboratory.

Preliminary steps have already been taken for a cooperative bluegrass breeding project between the Pennsylvania Agricultural Experiment Station (Mr. Musser) and the Laboratory.

A more or less verbatim report of the discussion that took place in the latter part of the afternoon, particularly that of visiting delegates, was made and distributed to those in attendance.

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